

A CHANGING INCLINATION OF ADK TOWARDS CYTOKININ ISOMERS DURING THE CELL CYCLE OF TOBACCO BY-2 CELLS

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ABSTRACT

Adenosine kinase (ADK), a component of the adenylate metabolic network and a key enzyme in the regulation of the intracellular level of adenosine (Ado), is also proposed to contribute to the metabolism of cytokinins. Through its potential role in conversion of cytokinin ribosides to nucleotides, ADK may be important in regulating the level of “active” cytokinins in plant cell. Recently, we have identified and characterized four *Nicotiana tabacum* (tobacco) BY-2 ADK isoforms: ADK1S, 1T, 2S and 2T. Our results suggested that some of tobacco ADKs may act specifically in the cell cycle G₂/M checkpoint, and through phosphorylation, rapidly reduce the increased content of active cytokinins to the basal level. The findings particularly point to ADK2S as an isoform potentially involved in cytokinin metabolism. In the following study, we demonstrate that *cis*-zeatin riboside (ZR) and iPA, in contrast to *trans*-ZR and DHZR, are the preferred cytokinin substrates for the recombinant tobacco ADKs (data not shown). Moreover, the conversion of *cis*-ZR to ZMP is reduced in a competitive manner by the presence of growing amount of *trans*-ZR and *trans*-Z, which itself cannot be subjected to phosphorylation (Figure 1).

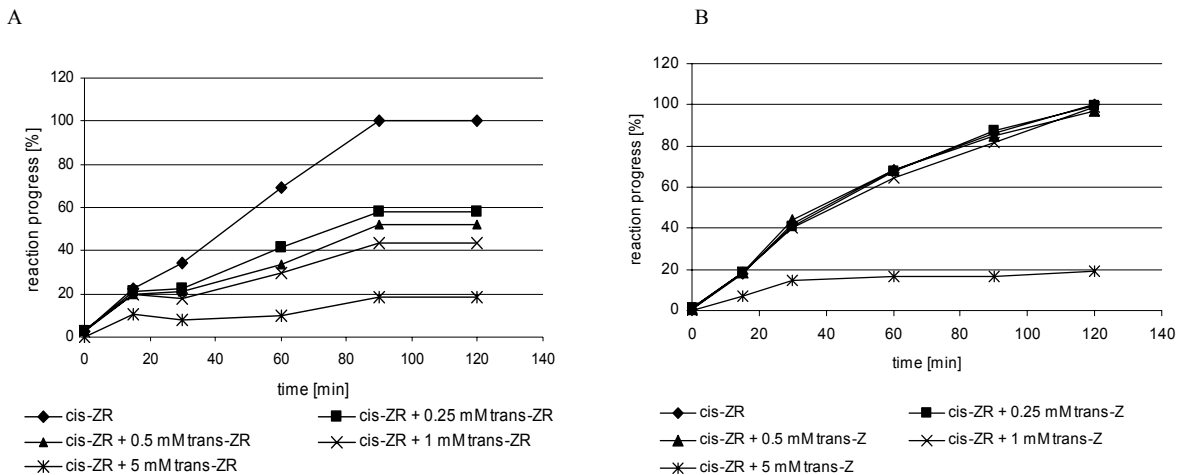


Figure 1: The conversion of *cis*-ZR to ZMP by tobacco ADK2S in the presence of growing amounts of (A) *trans*-ZR, (B) *trans*-Z, as measured by HPTLC analysis. The concentration of *cis*-ZR was 0.5 mM in each reaction. Formation of ZMP was monitored in each sample. Reaction progress is expressed as a percentage of the highest amount of product measured in the experiment.

Applying different substrates to incubation mixture containing ADK immunoprecipitates from each time point of the cell cycle of BY-2 resulted in different profiles in ADK activity. Activity towards Ado was the highest at the end of S phase with a second smaller peak appearing at 7 hours after aphidicolin release (figure 2A). When *cis*-ZR was used as the substrate, maximum activity visibly shifted towards M phase in a prolonged peak (figure 2B). The detection of a

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transient premitotic peak of *cis*-Z in BY-2 cells is in good agreement with the increasing amount of evidence on the presence and potential function of free *cis*-cytokinins in plants.

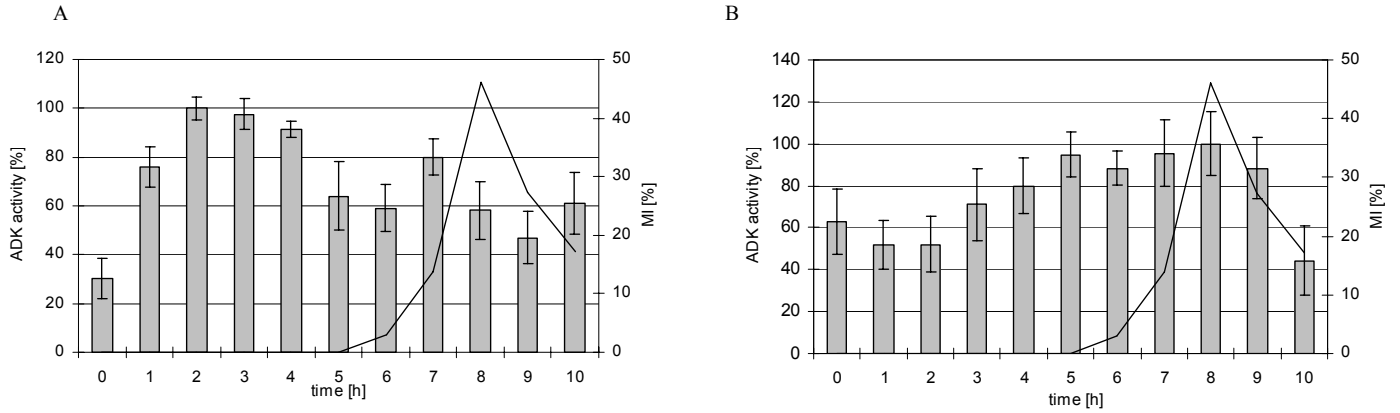


Figure 2: Analysis of ADK activity level during the tobacco BY-2 cell cycle in response to different substrates. The following substrates were used: *A*, 0.5 mM Ado; *B*, 0.5 mM *cis*-ZR. Bars correspond to ADK activity, line to mitotic index (MI). ADK activity is expressed as a percentage of total ADK at *A* - 2h, *B* - 8h time point. Graph values represent means \pm SE (n = 3).