

**THE REPRESSION OF SIX 14-3-3 ISOFORMS RESULTING IN THE
ACTIVATION OF NR AND SPS AND AN INCREASE IN THE
ANTIOXIDANT PROPERTIES OF TRANSGENIC POTATO PLANTS**

MAGDALENA ŻUK and JAN SZOPA

Institute of Biochemistry and Molecular Biology, University of Wrocław,
Przybyszewskiego 63/77, 51-148 Wrocław, Poland

Six 14-3-3 full-size cDNAs from potato plants were cloned and sequenced, and their high sequence homology was established. It was previously documented that the pattern of expression of 14-3-3 proteins in potato leaves was dependent on leaf maturity. Many recent reports pointed out the great importance of 14-3-3 proteins in plant metabolic pathways. It is suggested that members of this protein family affect nitrogen fixation by regulating nitrogen reductase, and carbohydrate metabolism by binding to sucrose phosphate synthase. In order to analyze the function of 14-3-3 in the potato plant, transgenic plants with repression of all the found isoforms were created. Thus-obtained plants were analyzed for sucrose phosphate synthase (SPS) and nitrate reductase (NR) activities, and for the products of these enzymes. A significant increase in NR activity (3 to 5 fold) was found in all the transgenic lines. It is suggested that 14-3-3 regulates NR activity *in vivo*, and this regulation is not isoform dependent. It was also calculated that 80% of NR activity may be 14-3-3 protein controlled. The increase in enzyme activity was complemented by exogenous 14-3-3 protein taken either from potato or *Cucurbita pepo*. The *in vitro* finding was confirmed in *in vivo* trials. But the glutamine, asparagine and protein contents only correspond to the enzyme activity increase in the case of one transgenic line G3.49. We also showed that there was a significant increase (5 to 9 fold) in SPS activity. The data obtained revealed that the repression of six 14-3-3 isoforms is more effective than single isoform repression for SPS activation, and that about 85% of its total activity is controlled by 14-3-3 proteins. This also suggests no specificity in the control of the enzyme by any one 14-3-3 isoform. As a consequence of the SPS increase, an increase in sucrose and subsequently in starch level was expected. The highest SPS activity in tubers from the G3.40 transgenic line resulted in a near 4 fold increase in the sucrose content, while all the other transgenic lines showed only slight changes in the sucrose quantity compared to the control value. A significant increase in the starch accumulation in the tubers of all the transgenic plants was noticed.

The decrease in 14-3-3 protein content resulted in an antioxidant capacity increase in the tuber extracts. Compared to the control, all the transgenic lines showed up to a 200 fold decrease in antioxidant potential (IC 50 – the amount of extracts inhibiting chemiluminescence deriving from luminol treatment with free radicals). A regulatory role of the 14-3-3 protein in antioxidant compound biosynthesis is suggested.