Dissertation Abstract

Changes in levels of several metabolites and changes in gene expression in response to UV-B radiation and other oxidative stresses were studied in plant tissues.

Intracellular levels of nicotinamide and trigonelline increased in pea (*Pisum sativum*) plants after exposure to UV-B radiation. The same result was seen in *Catharanthus roseus* tissue cultures after treatment with the free radical generating-chemicals AAPH and vanadylsulfate. Vanadylsulfate treatment increased both the phenylalanine ammonia lyase (PAL) activity and the content of reduced and oxidised glutathione in *C. roseus* tissue culture. The increases in PAL activity caused by AAPH or vanadylsulfate were prevented by 3-aminobenzamide, an inhibitor of the enzyme poly(ADP-ribose)polymerase. These results support the hypothesis [Berglund T., *FEBS Lett.* (1994) 351, 145-149] that nicotinamide and/or its metabolites may function as signal transductors in the response to oxidative stress in plants and that poly(ADP-ribose)polymerase has a function in the induction of defensive metabolism. At the same time, nicotinamide and trigonelline do not function as signalling compounds for CHS and PAL gene expression. Elevated nicotinamide and trigonelline levels occur in response to UV-B, but only at UV-B doses high enough to cause oxidative stress.

Pea protoplasts were used in deletion analysis of the parsley (*Petroselinum crispum*) CHS promoter. Introduction by electroporation of different parsley CHS-promoter/ß-glucuronidase(GUS)-reporter constructs into pea protoplasts leads to a high constitutive GUS expression and to the loss of the light-inducibility seen in the homologous parsley protoplast system. These results indicate that Unit 1 of the parsley CHS promoter is only partly responsible for the GUS expression detected. Instead, additional *cis*-elements, which are located downstream within 100 bp from the transcriptional start site, mediate the de-repression in pea protoplasts. In contrast, in yeast (*Saccharomyces cerevisiae*) cells, the GUS expression from the heterologous CHS/GUS construct is controlled by elements between Unit 1 and -100 bp. The results with pea protoplasts imply that protoplastation of pea leaf cells itself induces de-repression of CHS as a result of stress to the protoplasts. This notion was strengthened by the finding that mRNA levels of the endogenous chalcone synthase were drastically increased as the result of the protoplastation procedure.

Finally, molecular markers for different biochemical pathways (flavonoid biosynthesis, pathogen response) and for a short-chain alcohol dehydrogenase were used to study cell specificity of UV-B responses in *Argenteum* pea leaves. Epidermal and mesophyll tissues of irradiated pea leaves were examined separately in order to elucidate a detailed picture of gene expression and to compare the extent of DNA damage in these tissues.

Key words: UV-B, *Pisum sativum*, oxidative stress, nicotinamide, gene expression, signal transduction, chalcone synthase, *cis*-elements in parsley CHS-promoter, DNA damage.

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