Natural Inhibitors of Germination and Growth, VII
Synthesis of Ribulosebisphosphate Carboxylase in Darkness and Its Inhibition by Coumarin

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Z. Naturforsch. **49c**, 321–326 (1994); received March 10, 1994

Dedicated to Prof. Aloysius Wild on the occasion of his 65th birthday

Lepidium sativum, Cruciferin, Ribulosebisphosphate Carboxylase, Inhibition of Transcription

Cress *(Lepidium sativum)* seeds were germinated in darkness. Seedlings were investigated for soluble proteins by SDS-PAGE. Two proteins were identified by microsequencing: the small subunit of ribulosebisphosphate carboxylase (SSU) and the alpha subunit of the storage protein cruciferin. Net synthesis of small and large subunits of ribulosebisphosphate carboxylase (SSU and LSU) was investigated by Western blot. Net synthesis of both subunits was inhibited by coumarin. To the contrary, net synthesis of cruciferin was increased by coumarin. With specific cDNA probes, we determined steady state levels of the corresponding mRNAs (rbcS mRNA for SSU, rbcL mRNA for LSU). Both mRNAs can be detected in dry seeds; their amount increases during germination in the dark. Incubation with coumarin inhibits this increase. Inhibition of development by coumarin on the level of transcription is discussed.

Introduction

Coumarin (2H-1-benzopyran-2-one) is known since a long time as regulator of plant growth and development (review: Brown, 1981). Being produced by some plants, coumarin is listed as an allelophatic chemical (Putnam, 1983; Valio, 1973). In this connection, mainly its action as germination inhibitor has been considered (Williams and Hoagland, 1982; Reynolds, 1989). It inhibits root growth similar as several phenolic acids do (Glass, 1976). Tolerant species are believed to metabolize coumarin and detoxify it in this way (Sivan *et al.*, 1965). However, there are effects different from this inhibitory action: Svensson (1972) reported e.g. an increase of net DNA synthesis, decrease of net RNA synthesis and increase of the protein content per cell by coumarin in roots of maize and wheat. The lack of interaction of coumarin with several metabolic inhibitors led Svensson (1972) to the conclusion that coumarin effects already existing structures or enzymes which were not defined however.

In the course of our studies on protein patterns during germination of cress seeds, we characterized a seed protein as carboxy terminal fragment of the heat shock protein HSP70 (Oster *et al.*, 1992). This fragment occurs naturally in dry seeds but disappears normally during germination. Coumarin inhibits the degradation of this fragment. The opposite behaviour was found for a 17 kDa protein. We describe here its identification as the small subunit of ribulosebisphosphate carboxylase. This observation prompted a study on the accumulation of both subunits (SSU and LSU) of this enzyme on protein and mRNA level during germination of cress seeds in the dark and the influence of coumarin on these processes.

Materials and Methods

Treatment of plant material

Seeds of garden cress (*Lepidium sativum* L.) were either germinated with water ("water control") or treated with coumarin under otherwise identical conditions. Coumarin (final conc. 9×10^{-3} M) was applied to the filter paper as described before (Oster *et al.*, 1992). Cress seeds

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0939–5075/94/0500–0321 $ 03.00