Regulation of catalase synthesis during its light-induced turnover in leaves

M Schmidt, S Dehne, J Feierabend

Botanisches Institut, Goethe-Universität, D-60054 Frankfurt am Main, Germany.
Feierabend@em.uni-frankfurt.de, fax: 49-69 798 24822

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Introduction

The enzyme catalase detoxifies H₂O₂ which is produced in the photorespiratory pathway by the oxidation of glycolate in the peroxisomes. Although catalase provides an essential reaction of photoprotection, catalases are, however, generally light-sensitive. Catalases are oxidatively inactivated both in vitro and in vivo by blue light which is absorbed by their hemin cofactor. In leaves catalase may, in addition, be inactivated by reactive oxygens produced in the chloroplasts by photosynthetic reactions under high excitation pressure (Shang and Feierabend 1999). While the apoprotein of inactive catalase is degraded, the hemin cofactor was shown to remain intact (Feierabend and Dehne 1996). In mature leaves the loss of catalase is continuously replaced by de novo synthesis. While a constant steady state activity is thus maintained, the catalase protein has a rapid turnover in light (Hertwig et al. 1992). The rate of synthesis of new catalase, required for this turnover, must be continuously attuned to fluctuating light intensities. Mechanisms controlling catalase synthesis were investigated in mature leaves of rye. In mature rye leaves only one single catalase isozyme gene, designated at Cat1, is expressed (Schmidt and Feierabend 2000).

Materials and methods

Sections of 3 cm length were excised from mature primary leaves of 6-day-old rye seedlings (Secale cereale L.) grown at 96 µmol m⁻² s⁻¹ PAR. Leaf sections were incubated on nutrient solution for the times indicated (1 to 8 h) in either darkness, low light (LL) of 96 µmol m⁻² s⁻¹ PAR or high light (HL) of 500 µmol m⁻² s⁻¹ PAR (see Hertwig et al. 1992). After the incubations RNA or poly[A]⁺RNA was extracted from the leaf sections and analysed by Northern hybridizations or by cell-free in vitro translation in the presence of L-[³⁵S]-methionine with a wheat germ lysate. Incorporation into catalase in vitro was visualized by fluorography after immunoprecipitation with an antiserum against rye leaf catalase and subsequent SDS-PAGE (Hertwig et al. 1992).

Results

Changes of the contents of the transcript for the only major catalase isozyme CAT-1 were assayed by Northern hybridization after exposure of leaf sections from mature rye leaves to different light conditions (see methods). While previous investigations of the in vivo incorporation of L-[³⁵S]-methionine had shown that the apparent rate of catalase synthesis was about six times higher during a 7h exposure to high light than in leaf sections kept in darkness (Hertwig et al. 1992), the mRNA content did not increase with light intensity (Fig. 1a). By contrast, the mRNA content was even higher in darkness than in light-exposed leaves, suggesting that posttranscriptional mechanisms controlled the light-dependent rate of new
catalase synthesis during its light-induced turnover in mature leaves. In the leaves which maintained a constant level of catalase activity at high light intensity, CAT-1 was not replaced or complemented by the enhanced expression of any additional catalase isozyme. The transcript of an additional rye isozyme gene $Cat2$ which is expressed in roots (Schmidt and Feierabend 2000) and potential further catalase polypeptides that were observed in scutella, were not detected under the experimental conditions in the leaves. Also when the leaf catalase was experimentally completely inactivated by the inhibitor 3-aminotriazole, its mRNA did not increase (Fig. 1b). When the de novo synthesis of catalase was suppressed, e.g. by the presence of NaCl which inhibits translation, the steady state level of catalase greatly declined in rye leaves (Streb and Feierabend 1996). During catalase depletion in the presence of 0.2 M NaCl a marked increase of the $Cat1$ mRNA was observed (Fig. 1b). Incubations in 0.1-0.2 M NaCl in high light were also the only experimental conditions where a very low expression of rye $Cat2$ mRNA was induced (not shown) in mature green leaves.

Fig. 1. Northern analysis of changes of the transcript of the catalase gene $Cat1$ during various experimental treatments in sections of rye leaves grown in low light (Control, 0h). a) Leaf sections were for 7h exposed to low light (LL), high light (HL) or darkness. b) Leaf sections were incubated in high light in the presence or absence of 2 mM 3-aminotriazole (AT). c) Leaf sections were 24h incubated in high light in the presence of NaCl. 20 µg total RNA was applied per lane.

In normal mature rye leaves posttranscriptional mechanisms must be active to attune the rate of catalase synthesis to the rate of its photodegradation, since its mRNA content did not vary in proportion to the light intensity. To study potential translational controls catalase synthesis was investigated in vitro with poly[A]$^+$ RNA from rye leaves in a cell-free wheat germ lysate. In vitro incorporation of $^{35}$S-methionine was assayed by fluorography after immunoprecipitation and SDS-PAGE. The rate of catalase synthesis in vitro was greatly stimulated by the addition of hemin up to an optimum between 40-80 µM (depending on the wheat germ preparation), and it was reduced when the endogenous hemin of the wheat germ extract was captured by the addition of apoperoxidase. A similar dependence on hemin was described for catalases A and T of Saccharomyces cerevisiae (Hamilton et al. 1982). Since the hemin cofactor of catalase was not damaged during photoinactivation (Feierabend and Dehne
1996), hemin may act as an important factor to adjust the rate of catalase synthesis in leaves to the extent that hemin is liberated from inactivated catalase in light.

An unexpected observation was that the translational efficiency of the Cat1 mRNA in a wheat germ translation assay was modulated by the light conditions to which the rye leaf sections had been exposed, prior to extraction of the poly[A]+ RNA. After dark exposure translation of catalase was very low, although its mRNA content was high, relative to light incubations. The translational efficiency for catalase increased with the light intensity to which the leaves had been exposed, prior to RNA extraction. The light-modulated changes of translational activity were observed both in the absence and presence of hemin. Light/dark-induced changes of the translational efficiency of the catalase mRNA were reversible and independent of RNA transcription (Fig. 2). After 5h in high light the translational activity declined during a subsequent dark exposure with a half time of 2h, whereas the amount of Cat1 mRNA increased. After transfer from darkness to high light the translational activity increased after a lag of 1h. This light-induced increase of the translational activity occurred also in the presence of cordycepin (not shown), indicating that it did not require the synthesis of a new active mRNA, but a preexisting mRNA appeared to be activated. A comparable translational activation of a catalase mRNA (Cat2 of maize) was observed when etiolated maize leaves were exposed to light (Skadsen and Scandalios 1987).

![Fig. 2. Changes of the amounts and of the translational activity of catalase mRNA after transfer of leaf sections from high light to darkness (left) or from darkness to high light (right). Incorporation into the catalase polypeptide was assayed after in vitro translation of poly[A]+RNA in a wheat germ extract in the presence of 30 µM hemin.](image)

Catalase mRNA that was transcribed in vitro from a rye Cat1-cDNA clone was translated in the wheat germ lysate only when it was capped with a monomethylated cap analogue (m7GpppG). When the cap was unmethylated or doubly methylated the mRNA was not efficiently translated. The results suggest that cap methylation may play an important role for the translational activity of the Cat1-mRNA.

**Conclusions**

The rate of catalase synthesis during its light-induced turnover in mature rye leaves is determined by posttranscriptional/translational mechanisms. Catalase synthesis is controlled by the availability of the hemin cofactor. The amount of hemin liberated from inactivated catalase which is proportional to the light intensity may control the extent of new catalase
synthesis in leaves. Furthermore, catalase synthesis is controlled by light-modulated changes of the translational activity of its mRNA. The translational efficiency of catalase mRNA of rye leaves is reversibly activated by light in a dose-dependent manner and inactivated in darkness. Such changes of the translational efficiency must result from some modification of the catalase mRNA.

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References