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Research article

Partial characterization and expression of leaf catalase in the CAM-inducible halophyte *Mesembryanthemum crystallinum* L.

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Abstract

Catalase (CAT; EC 1.11.1.6) isolated from leaves of the halophytic plant *Mesembryanthemum crystallinum* is characterized by a high apparent molecular mass of about 320 kDa, and high resistance to denaturing agents (10% ME). SDS-treatment breaks active oligomeric CAT into the less active and putatively dimeric form of 160 kDa apparent molecular mass. Three subunits are resolved after denaturing PAGE: 79, 74 and 62 kDa. Higher molecular masses of subunits coincide with increased activity of CAT. *M. crystallinum* leaf CAT reveals a diel variation in the resistance to denaturing factors and the stability of CAT is increased in a light-dependent manner both in C_3- and in CAM-induced plants. Unchanged level of leaf CAT transcripts is documented in the diurnal cycle of C_3 plants and after salinity-induced crassulacean acid metabolism (CAM).

Keywords: Catalase; Crassulacean acid metabolism; Molecular mass; *Mesembryanthemum crystallinum*; Salinity; Subunits

1. Introduction

Catalase (CAT, H_2O_2:H_2O_2 oxidoreductase; EC 1.11.1.6) by scavenging hydrogen peroxide to water and oxygen is an important component of cell defense mechanisms against oxidative stress. As a part of the antioxidant response system, CAT plays a role in maintaining the redox homeostasis of the cell. It is also involved in the processes of signal transduction between the cells and so-called 'systemic acquired acclimation' due to the signalling action of H_2O_2 [11,12,27].

This enzyme is present in all aerobic organisms and many anaerobic. In plants, multiple isoforms of this enzyme are usually present, and they are expressed in different tissues and developmental stages [3,17,23,32,42]. In green leaves a majority of CAT activity is associated with peroxisomes [12]. However, also a mitochondrial form has been documented in maize [32], and a chloroplastic form in spinach [35]. A comparison of CATs reveals a wide range of biochemical activities and kinetic properties [41]. There are indications for both transcriptional and posttranscriptional control of CAT activity [3,23,32,28,39,40,42,45]. CATs are predominantly heme-containing tetrameric proteins of about 240 kDa molecular mass [32]. However, some exceptions are: (1) non-heme CATs [41,44], (2) dimeric CAT [31], and (3) CATs of much higher molecular mass [5,8,15].

In green leaves CAT mainly acts as a sink for H_2O_2 generated during photorespiration [6,43]. Therefore, it is reasonable to speculate that intensity of photorespiration may influence the activity of CAT. Crassulacean acid metabolism (CAM) performing plants seem to be a good model for studying mechanisms of CAT activity regulation, since they are subjected to strong fluctuations of intracellular CO_2 and O_2 levels during the day, which presumably affect photorespiration [22,37]. CAM is a specialized mode of photosynthetic carbon acquisition, in

Abbreviations: 3-AT, 3-amino-1,2,4-triazole; au, arbitrary units; BCIP, 5-bromo-4-chloro-3-indolyl phosphate; CAM, crassulacean acid metabolism; ME, β-mercaptoethanol; NBT, nitro blue tetrazolium; p*I*, isoelectric point; TEMED, *N*,*N*,*N*′,*N*′-tetramethylethylenediamine; Tricine, *N*-tris[hydroxymethyl] methylglycine.

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which CO_2 is taken up at night via the enzyme phospho-*enol*pyruvate carboxylase (PEPC), and the products of dark carboxylation (i.e. organic acids) are subsequently decarboxylated during the day, thereby elevating [CO_2] around Rubisco behind closed stomata [10]. Indeed strong diurnal fluctuations of CAT activity were detected after a C_3–CAM transition in *Mesembryanthemum crystallinum* leaves, which might indicate modulations of photorespiratory flux [29]. In the halophytic plant *M. crystallinum* a C_3–CAM metabolic shift is dependent mainly on the environmental events and may be induced by a broad range of stress factors, among them salinity being routinely used [1,10,26]. It is also well established that CAT activity is associated with plant resistance to salinity and drought. Numerous data documented that salinity negatively affects synthesis and activity of CAT [9,13,38]. In this respect, catalases of the halophytic plants seem to be more resistant [16]. However, the reason for this has not been clarified yet. Therefore, the aim of this study was to characterize leaf CAT from the halophytic *M. crystallinum* plants. The second goal of this work was to investigate the mechanism of CAT activity fluctuations, which appear in CAM-induced plants.

2. Results

Two bands of catalase activity were revealed by native gel electrophoresis in extracts of soluble proteins from *M. crystallinum* leaves (Fig. 1a). A major catalase form (CAT-1) was totally inhibited by 20 mM 3-AT (Fig. 1a). In contrast, the second slower migrating band was affected only slightly, thus, we consider this form as an aggregate exhibiting both CAT and MnSOD activities. CAT activity of such aggregated form was not visualized before. However, the presence of a high-mass MnSOD-like form in *M. crystallinum* was previously documented by SOD activity staining [36].

According to native gradient PAGE the apparent molecular mass of CAT-1 is about 320 kDa (Fig. 1b). When non-denatured proteins were loaded onto SDS-gel one band of catalase activity of 160 kDa apparent molecular mass was resolved (Fig. 1c), which possibly represented an active CAT dimer.

After FPLC separation on a Superose column the majority of catalase activity was found at 38 min of elution time (Fig. 2a). This corresponded to the molecular masses in the range of 257–328 kDa (Fig. 2b), as calculated from calibration of the column by gel filtration standards, which is in agreement with the data obtained by gradient electrophoresis. The apparent molecular mass of CAT/MnSOD aggregate determined by FPLC size exclusion was in the range of 520–630 kDa, and of 610–650 kDa by mean of the native gradient PAGE (data not shown).

The predominant catalase form (CAT-1) was resistant to the strong reducing agent 10% ME, whilst, a putative aggregate band disappeared in this treatment (Fig. 3). High resistance of CAT-1 to ME suggests that its subunits may not be linked by disulphide bonds. Whereas, an aggregation might be dependent on the oxidative cross-linking, which seems to be more intensive towards the end of the photoperiod with increasing oxidative load.

Immunoblotting after mild-denaturation of protein samples (see description in: *M & M* Section 4.3.) using human CAT antiserum revealed three forms of CAT (79, 74 and 62 kDa) in experiments with proteins extracted at different time-points of the diel cycle (Fig. 4). In extracts from C_3 plants 74 kDa form appeared with the onset of light and disappeared about 6 h into the dark phase, while polypeptide of 62 kDa was detected only at the end of darkness and at the beginning of the light period. A 74 kDa form was accompanied by the faint band of 79 kDa molecular mass for most of the daytime. Although, it is not clear whether this band represents another

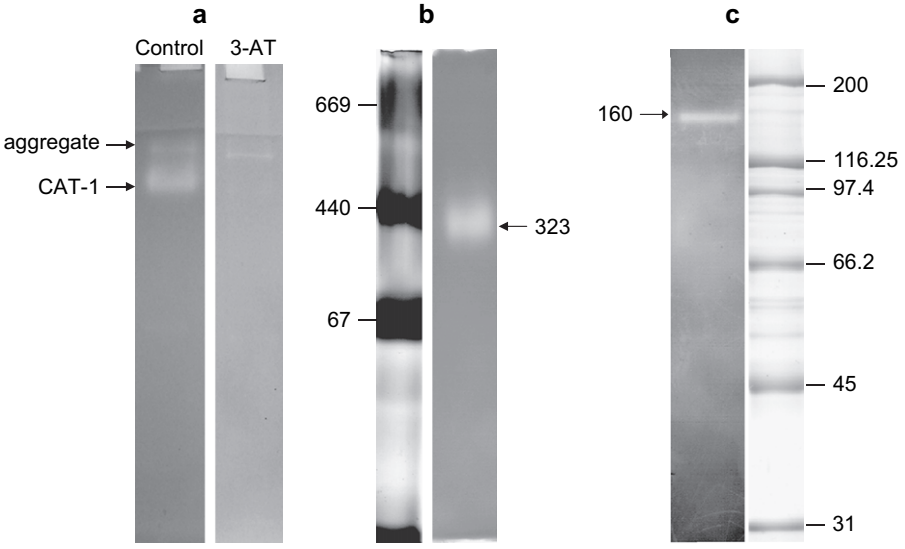

Fig. 1. Catalase activity in extracts of leaves of *M. crystallinum* C_3 plants visualized by the CAT activity staining after: (a) native PAGE, (b) native gradient PAGE, and (c) SDS-PAGE of non-denatured protein extracts. The amount of 10 (a), 20 (b) and 40 μg (c) of soluble protein was loaded. Gel filtration standards (b) and SDS-broad range standards (c) were used; values represent molecular masses in kDa. Presented gels are typical examples of at least six independent electrophoretic runs.