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ENVIRONMENTALLY CONTROLLED CHANGES OF PHOSPHOENOLPYRUVATE CARBOXYLASES IN *MESEMBRYANTHEMUM*

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Key Word Index—*Mesembryanthemum crystallinum*; Mesembryanthemaceae; Crassulacean acid metabolism; phosphoenolpyruvate carboxylase, gel electrophoresis; NaCl treatment.

Abstract—NaCl treated *Mesembryanthemum crystallinum* plants exhibit a Crassulacean acid metabolism. The activity of phosphoenolpyruvate (PEP) carboxylase, the enzyme responsible for CO_2 dark fixation, depends on leaf age showing maximum activity in mature leaves. Electrophoresis revealed that the young leaves possess only two protein bands with PEP carboxylase activity, while older leaves have 3 bands. The removal of NaCl from the soil resulted in the disappearence of the 3rd band obtained after electrophoresis and a decline in the total activity of the PEP carboxylase. The reintroduction of NaCl at the same concentration as before did not restore the activity of the PEP carboxylase nor did it restore the initial electrophoretic band pattern.

INTRODUCTION

Mesembryanthemum crystallinum belongs to a group of plants which respond to salination by the development of a Crassulacean acid metabolism (CAM) [1–3]. This process is controlled by plant and leaf age (v. Willert *et al.*, in preparation) and is accompanied by characteristic changes in the activity and the properties of PEP carboxylase [4, 5]. Until now no information is available on what happens to the PEP carboxylase when salt treated plants exhibiting a Crassulacean acid metabolism are returned to non saline conditions, a process associated with the disappearance of CAM features.

RESULTS

At the end of a salt treatment (300 mM NaCl) lasting 37 days *M. crystallinum* plants exhibit CAM i.e. net CO_2 uptake and malate synthesis in the dark. The activity of PEP carboxylase, the enzyme responsible for CO_2 fixation in the dark [6], was high in fully expanded leaves but declined rapidly with decreasing leaf age (Fig. 1A). In the youngest leaf less than 1/15 of the activity in the mature leaves was present. The ability of the leaves to accumulate malate at night corresponded with the activity of the PEP carboxylase. The amount of accumulated malate declined from about 530 μmol/g dry wt in the 3rd, 4th, and 5th leaf pair to 180 μmol in the 6th and 15 μmol in the youngest leaf pair.

Preliminary experiments were done to assure that the differences in PEP carboxylase activity did not simply result from a variable activation or inactivation caused by endogenous organic or inorganic ions which vary with leaf age. Recently Pi was shown to regulate the activity of PEP carboxylase prepared from *M. crystallinum* [7]. Our experiments demonstrated that a 50-fold dilution is sufficient in lowering the concentration of known endogenous effectors so that they could no longer influence enzyme activity. The results presented in this paper were obtained with a 100-fold dilution.

Removal of NaCl from the soil resulted in a considerable decrease in the total activity of PEP carboxylase in all but the top leaf pair (Fig. 2). Within one day the activity of the enzyme declined to about one third of its initial activity with a slight subsequent decrease. The reintroduction of NaCl at the same concentration as before did not restore the activity of the PEP carboxylase which remained at a low level for the following 8 days. During the course of the experiment the CO_2 gas exchange of the plants was measured. At each day a net uptake of CO_2 was noted indicating that the plants did not suffer from the treatments.

An electrophoretic separation on acrylamide gels performed with crude extracts of the different leaves of a

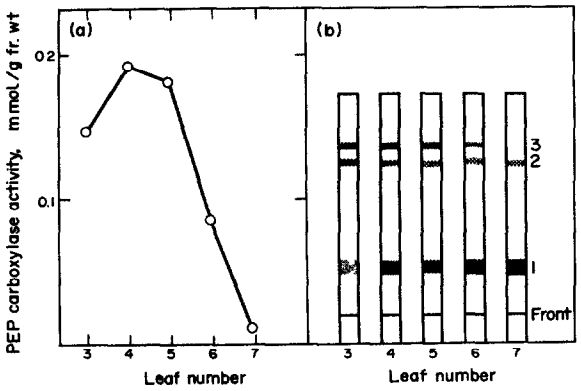

Fig. 1(a). Activity of the PEP carboxylase in the different leaves of saline grown (300 mM NaCl) *M. crystallinum* plants. Leaves are numbered following decreasing leaf age. The enzyme activity was measured in crude extracts. (b) Bands with PEP carboxylase activity obtained after disc electrophoresis and specific staining for PEP carboxylase. Aliquots of the crude extracts from a. were directly subjected to gel electrophoresis. Stippling intensity represents stain intensity (= enzyme activity).

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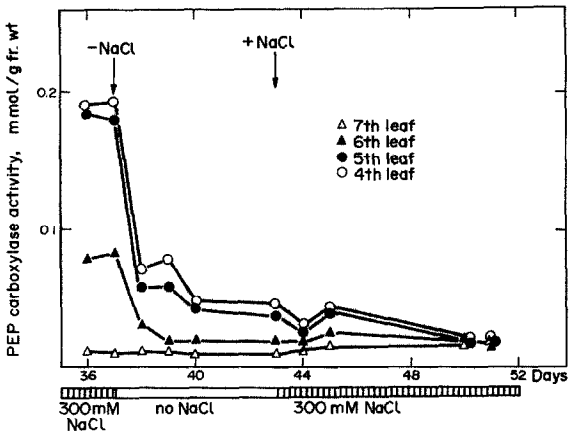

Fig. 2. Changes in the activity of the PEP carboxylase in the different leaves of *M. crystallinum* following the removal of 300 mM NaCl from the soil and subsequent reintroduction of NaCl (300 mM). Leaves are numbered following decreasing leaf age.

CAM exhibiting *M. crystallinum* plant led to the detection of several bands with PEP carboxylase activity. The results are given in Fig. 1b and may be compared with the sp act of this enzyme in the same crude extracts (Fig. 1a). The relative intensities of the bands varied considerably among the different leaves. Young leaves contrasted sharply with mature leaves in both the level of activity and the number of bands. Three bands were detected in mature leaves as compared with only two in the youngest one. The additional 3rd band in the mature leaves had the lowest mobility indicating that this PEP carboxylase fraction was probably the largest of the 3 proteins with PEP carboxylase activity, and could be aggregated state of either newly synthesized or pre-existing subunits. Prior to the removal of NaCl from the soil the 3rd band was most intense. After 24 hr it was the least intense and could not be detected in preparations of the following days. The pattern of the bands did not change after reintroduction of NaCl. Thus, neither the activity of the PEP carboxylase nor the electrophoretic fraction pattern returned to the initial state.

DISCUSSION

In an earlier report we demonstrated that NaCl-promoted development of a CAM in *M. crystallinum* is correlated with the appearance of a 3rd electrophoretic band of the PEP carboxylase [8]. The present results confirm the previous conclusion that this band is responsible for the high activity of the PEP carboxylase in *Mesembryanthemum* plants exhibiting CAM [5]. The decline in the activity of PEP carboxylase coincides with the loss of the 3rd band.

Some further deductions can be made from our results. After removal of NaCl from the soil none of the NaCl taken up during salt treatment is exported from the leaves and only a slight dilution by increased water uptake occurred [9]. Hence, the reactions leading to the synthesis of the 3rd band with PEP carboxylase activity cannot depend on the presence of Na^+ and (or) Cl^- but must be mediated by another factor. On the other hand, the reintroduction of NaCl at the same concentration to which the plants had previously become adjusted did not cause the same reactions as in non-saline plants. Thus the pretreatment of the plants as well as leaf age is important in the regulation of the 3rd band of PEP carboxylase activity.

The rapid disappearance of the additional PEP carboxylase band provides good evidence for a high turnover rate and a short half life time of this fraction. Both properties seem to be prerequisites which facilitate regulation of enzyme capacity. If the synthesis is stopped by changed environmental conditions the total activity falls and the additional enzyme fraction disappears, indicating that PEP carboxylase of *M. crystallinum* is under environmental control.

EXPERIMENTAL

M. crystallinum plants were grown initially in a greenhouse and were transferred to a temp. and light controlled chamber at an age of 6 weeks. Conditions in the chamber were 20 klx incandescent white light, 25° during the day and 18° during the night with a photoperiod of 12 hr. 3 weeks later some plants were irrigated with 300 mM NaCl. After 37 days, salt treatment was stopped by rinsing the plants thoroughly with H_2O and nutrient soln [10] for 6 hr. After that time and on the following days Na and Cl content in the soil was determined and found not to differ significantly from the content in the soil of control plants. At the end of the first salt treatment, during the non-saline period, and during the subsequent salt treatment, the activity of PEP carboxylase in the different leaves was determined and the enzyme electrophoretically fractionated on polyacrylamide gels. The leaves were harvested at the end of the light period, when they had the lowest content of the enzyme inhibitor malate, and homogenized in a mortar with their own wt of buffer which contained Tris–bicine (200 mM, pH 8.5) and $MgCl_2$ (5 mM). The homogenate was filtered and centrifuged at 45000 *g* for 10 min. The resulting supernatant was used immediately for enzyme assay and disk electrophoresis. PEP carboxylase (EC 4.1.1.31) activity was assayed at 30° by coupling the reaction with malate dehydrogenase (EC 1.1.1.37). The oxidation of NADH was measured at 340 nm. The assay system contained Tris–bicine buffer (100 mM, pH 7.5), PEP (1 mM), NADH (0.15 mM), $NaHCO_3$ (5 mM), $MgCl_2$ (2 mM), and crude extract in a total vol. of 2 ml. The polyacrylamide disc electrophoresis technique of refs [11] and [12] was used to analyze the crude extracts. Gels (6%) were at pH 7.5 and the buffer at pH 7. The run was performed with 5 mA per gel. The procedure for the specific staining of PEP carboxylase was a slightly modified technique of ref [13]. Gels were incubated in a soln which contained PEP, $NaHCO_3$, $MgCl_2$ and fast violet B the specific stain for oxaloacetic acid.

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