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Preferential accumulation of betaine uncoupled to choline monooxygenase in young leaves of *sugar beet* – Importance of long-distance translocation of betaine under normal and salt-stressed conditions

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Summary

It has been reported that glycinebetaine (betaine) is synthesized in response to abiotic stresses via a two-step oxidation of choline in which choline monooxygenase (CMO) and betaine aldehyde dehydrogenase (BADH) are involved. Here we show that significant amounts of betaine, $>20\,\mu mol/gFW$, accumulated in young leaves of *Beta vulgaris* even under normal growth conditions, whereas levels in old leaves, cotyledons, hypocotyls, and roots were low. Under the same conditions, CMO accumulates exclusively in old leaves and is difficult to be detected in young leaves. By contrast, the levels of BADH were high in all tissues. Exogenously supplied choline was converted into betaine in old leaves, but levels were significantly lower in young leaves under the same conditions. When d_{11}-betaine was applied exogenously to old leaves, it was translocated preferentially into young leaves and roots. In response to salt stress, betaine levels increased in all tissues, but most significantly increased in young leaves. The levels of CMO increased in various tissues, but were low in young leaves. A betaine transporter gene was isolated. Its expression was more strongly

Abbreviations: A. halophytica, Aphanothece halophytica; BADH, betaine aldehyde dehydrogenase; BetT, betaine transporter; C, cotyledons; CBB, Coomassie Brilliant Blue; CDH, choline dehydrogenase; CMO, choline monooxygenase; GFP, green fluorescent protein; H, hypocotyls; L, leaves; MES, 2-(*N*-morpholino)ethanesulfonic acid; ProT, proline transporter; R, roots; TM, transmembrane.

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induced in old leaves than in young leaves. Based on these data, we discussed the role of CMO and betaine transporter under stress and non-stress conditions.

Introduction

Many bacteria, plants, and animals accumulate glycine betaine (betaine) under water or salt-stress conditions. In most organisms, betaine is synthesized by a two-step oxidation of choline: choline → betaine aldehyde → betaine (Rathinasabapathi et al., 1997; Takabe et al., 2006). In plants, the novel rieske-type iron–sulfur enzyme choline monooxygenase (CMO) catalyzes the first step (Burnet et al., 1995; Rathinasabapathi et al., 1997), whereas membrane-bound choline dehydrogenase (CDH) or soluble choline oxidase (COX) catalyzes the first step in animals and bacteria (Takabe et al., 2006). CMO so far has been found only in Chenopodiaceae and Amaranthaceae, but not in some betaine-accumulating plants such as mangrove (Russell et al., 1998; Hibino et al., 2001). The same enzyme, betaine aldehyde dehydrogenase (BADH), performs the second step in plants, animals, and bacteria. In some bacteria, such as a halotolerant cyanobacterium, betaine is synthesized from glycine by a series of three-step methylation reactions (Waditee et al., 2003).

Although the regulation of betaine synthesis is important, its mechanisms are poorly understood. This is partly due to the difficulty of transforming betaine-accumulating plants such as spinach, barley, and mangrove. Hitherto, it has been shown that the levels of CMO mRNA, protein, and enzyme activity were induced in response to abiotic stresses such as salt and drought in spinach (Rathinasabapathi et al., 1997), sugar beet (Chenopodiaceae), and *Amaranthus caudatus* (Amaranthaceae; Russell et al., 1998). It was also shown that on the removal of drought stress, the levels of CMO mRNA and protein decreased to their original levels (Russell et al., 1998). Very recently, CMO was detected in the seeds of sugar beet (Catusse et al., 2008). Little is known regarding other molecular mechanisms of betaine synthesis.

Few studies have reported on the transporters for betaine in plants. We isolated the betaine transporter genes from betaine-accumulating mangrove *Avicina marina* (Waditee et al., 2002). It was shown that the expression and activity of betaine transporters were induced by salt stress, and that they transport proline as well as betaine (Waditee et al., 2002). Homologous transporters from tomato and *Arabidopsis*, betaine non-accumulators, have been shown to transport betaine and proline (Schwacke et al., 1999; Grallath et al., 2005), although the corresponding transporters from rice and barley do not transport proline or betaine (Igarashi et al., 2000; Ueda et al., 2001).

Extensive studies on the accumulation of betaine in betaine-non-accumulating plants by exogenous apply of betaine or genetic manipulation of betaine synthesis gene have been carried out (Chen and Murata, 2002, 2008; Rontein et al., 2001). But, the engineered betaine levels are very low, 0.3–1.0 μmol/gFW and improvements of abiotic stresses have been shown with a limited success. By contrast, accumulation levels of betaine in betaine-accumulating plants are often higher than 30 μmol/gFW. This indicates that some unknown factor(s) limit the accumulation of betaine in betaine-non-accumulating plants. Therefore, it is interesting to study the molecular mechanisms of betaine accumulation in betaine-accumulating plants.

Sugar beet is a betaine-accumulating dicotyledonous plant of the Chenopodiaceae family that has high economic value because it is one of the two main sources of sucrose (Catusse et al., 2008). In addition to sucrose, a large amount of betaine accumulates in the tap roots of sugar beet (Russell et al., 1998). Here, we examined the levels of betaine and CMO in various organs of betaine accumulator. Surprisingly, we found that significant amounts of betaine accumulate in young leaves without the accumulation of CMO, even under control conditions. A betaine transporter gene was isolated. Its expression was more strongly induced in young leaves than in old leaves. In addition to a protective role of betaine, its role on the growth of actively developing cells under normal conditions was discussed.

Materials and methods

Plant materials

Sugar beet (*Beta vulgaris* L., cv. NK-219 mm-0) was used throughout this study. The seeds were germinated and grown on soil containing vermiculite with 1/10 MS solutions (130 mL) in a growth chamber with a 16-h light (25 °C, 100 $\mu E\,m^{-2}\,s^{-1}$)/8-h dark (20 °C) cycle and 60% relative humidity, unless otherwise stated. One-month-old *B. vulgaris*