Mini-Review

MARINE BIOTECHNOLOGY

Salt-Regulated Mannitol Metabolism in Algae

Koji Iwamoto, Yoshihiro Shiraiwa

Functional Biosciences, Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan

Received: 12 March 2005 / Accepted: 28 April 2005 / Online publication: 8 August 2005

Abstract

Mannitol, one of the most widely occurring sugar alcohol compounds, is found in bacteria, fungi, algae, and plants. In these organisms the compound acts as a compatible solute and has multiple functions, including osmoregulation, storage, and regeneration of reducing power, and scavenging of active oxygen species. Because of the diverse functions of mannitol, introducing the ability to accumulate it has been a hallmark of attempts to generate highly salt-tolerant transgenic plants. However, transgenic plants have not yet improved significantly in their salt tolerance. Recently, we purified and characterized 2 enzymes that biosynthesize mannitol, mannitol-1-phosphate dehydrogenase (M1PDH) and mannitol-1-phosphate-specific phosphatase, from the marine red alga *Caloglossa continua*, which grows in estuarine areas where tide levels fluctuate frequently. The activation of *Caloglossa* M1PDH is unique in that it is regulated by salt concentration at enzyme level. In this review we focus on the metabolism of mannitol, mainly in marine photosynthetic organisms, and suggest how this might be applied to producing salt-tolerant transgenic plants.

Key words: algae — halotolerant — mannitol metabolism — mannitol dehydrogenase — mannitol-1-phosphatase — salt-regulation

Introduction

Mannitol, a 6-carbon acyclic sugar alcohol, is one of the most abundant polyols occurring in nature (Stoop et al., 1996). It is synthesized in a diverse group of organisms, including bacteria (Wisselink et al., 2002), fungi (Jennings, 1984), apicomplexa (Schmatz et al., 1989), algae (Ben-Amotz and Avron, 1983; Kremer and Kirst, 1982), lichens (Armstrong and Smith, 1998), and higher plants (Bieleski, 1982). In many photosynthetic organisms mannitol is synthesized as a major primary photosynthetic product (Yamaguchi et al., 1969) and is used as an important translocatory (Schmitz and Srivastava, 1975) and storage compound (Kremer and Willenbrink, 1972). In these organisms mannitol has important physiologic functions owing to its biologically important properties, such as high solubility and high compatibility with organic macromolecules. Here we describe the biological significance of mannitol and its metabolism in various organisms.

Biological Significance of Mannitol

One of the most important physiologic functions of mannitol is the control of cell turgor: mannitol increases in intracellular concentration at low water activity, as in hypertonic conditions (Karsten et al., 1997a; Yancey et al., 1982; Davison and Reed, 1985). In highly saline environments mannitol accumulation was observed in several brown algae (Munda, 1964; Reed et al., 1985), the red alga *Caloglossa leprieurii* (Karsten et al., 1994; Mostaert et al., 1995a), the prasinophycean alga *Platymonas suecica* (Hellebust, 1976), several fungi (Allaway and Jennings, 1970; Jennings, 1984; Stoop and Mooibroek, 1998), and a higher plant, *Apium graveolens* (Stoop and Pharr, 1994). Some parasitic flowering plants, such as witchweed and broomrape, enhance their osmolarity by accumulating mannitol, thus enabling the parasites to absorb water and nutrients from their host plants because of the difference in water potential (Simier et al., 1998; Robert et al., 1999; Delavault et al., 2002).

Mannitol may also function as an antioxidant owing to its ability to scavenge free radicals (Tandon et al., 2003; Yu et al., 2003). Smirnoff and Cumbers (1989) showed that mannitol rescued the hydroxylation of salicylate and denaturation of malate dehydrogenase from the hydroxyl radical, a most potent oxidant in vivo. Some fungal phytopathogens also accumulate mannitol for neutralizing the high

---

Correspondence to: Yoshihiro Shiraiwa; E-mail: emilhux@biol.tsukuba.ac.jp

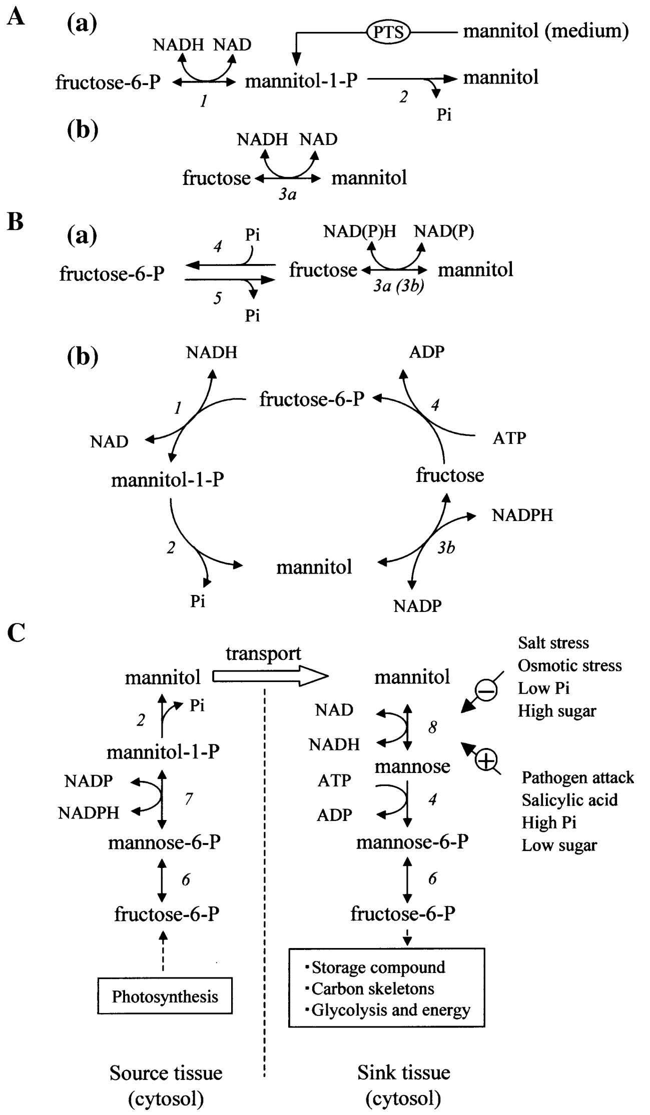

Fig. 1. Metabolic pathway of biosynthesis and degradation of mannitol. **A:** Proposed mannitol metabolism in non-lactic-acid bacteria and homofermentative-lactic-acid bacteria (a) and heterofermentative-lactic-acid bacteria (b). **B:** Proposed mannitol metabolism in fungi (modified from in Wisselink et al., 2002). **C:** Proposed mannitol biosynthesis and catabolism in source (left) and sink (right) tissues of higher plants (modified from Stoop et al., 1996). PTS indicates phosphoenolpyruvate-dependent mannitol phosphotransferase system; 1, mannitol-1-phosphate dehydrogenase (M1PDH); 2, mannitol-1-phosphatase (M1Pase); 3a, mannitol 2-dehydrogenase (M2DH); 3b, NADP-dependent M2DH; 4, hexokinase; 5, sugar phosphatase; 6, mannose-6-P isomerase; 7, mannose-6-P reductase; 8, mannitol 1-dehydrogenase (M1DH). Table 1 lists the isolation and gene registration of these enzymes. Circled minus and plus symbols, down- and upregulation of the reaction.

amounts of reactive oxygen species produced by plants in response to pathogen attacks (Apostol et al., 1989; Joosten et al., 1990; Jennings et al., 1998; Keller et al., 1998). Interestingly, to combat the fungal defense system, some infected plants synthesize mannitol 1-dehydrogenase (M1DH) de novo to degrade the phytopathogen-originated mannitol, even though the plants do not possess the pathway for mannitol synthesis (Williamson et al., 1995, 2002; Stoop et al., 1996). The infectant protection conveyed by M1DH was confirmed by a transgenic tobacco plant that expressed celery M1DH and showed high resistance to the mannitol-secreting fungal pathogen *Alternaria alternata* (Jennings et al., 2002). The radical scavenging capacity of mannitol was demonstrated more directly by Shen et al. (1997a), who reduced oxidative damage by hydroxyl radicals in a transgenic plant by introducing *mtlD*, the mannitol-1-P dehydrogenase (M1PDH) gene of *Escherichia coli*, into chloroplasts. The authors further showed that mannitol could shield susceptible thiol-regulated enzymes, such as phosphoribulokinase, from inactivation caused by hydroxyl radicals in the plant (Shen et al., 1997b). More recently, Abebe et al. (2003) suggested that the performance of a mannitol-accumulating transgenic plant improved because of the scavenging of reactive oxygen, rather than osmoregulatory effects, as the plant did not accumulate sufficient mannitol to sustain the osmotic potential. Fig. 1 summarizes the metabolic pathways for mannitol, and Table 1 lists the enzymes and genes with their accession numbers.

Mannitol Metabolism in Non-photosynthetic Organisms

In bacteria 2 different species-dependent biosynthetic pathways have been elucidated (Wisselink et al., 2002; Fig. 1, A). In non-lactic-acid bacteria and homofermentative-lactic-acid bacteria, mannitol is synthesized from fructose-6-P via mannitol-1-P by M1PDH and mannitol-1-phosphatase (M1Pase, 3.1.3.22; Fig. 1, A (a); Table 1). However, this pathway is commonly used to degrade, not biosynthesize, mannitol to produce energy. Mannitol imported as a carbon source is converted to mannitol-1-P by a phosphoenolpyruvate-dependent specific phosphotransferase system. The mannitol-1-P is oxidized by M1PDH to fructose-6-P and then catabolized via the glycolytic pathway (Fig. 1, A (a)). In contrast, heterofermentative-lactic-acid bacteria produce mannitol as a result of fructose uptake and utilization, and mannitol is directly synthesized from fructose by mannitol 2-dehydrogenase (M2DH) without the synthesis of mannitol-1-P (Fig. 1, A (b); Table 1).

In fungi 2 metabolic pathways for mannitol have been reported. In *Agaricus bisporus*, shiitake mushrooms, and *Dendryphiella salina*, fructose is gener-

Table 1. Enzymes Involved in Mannitol Metabolism in Bacteria, Fungi, Algae, and Higher Plants

No.	*Enzyme name (EC number)*[a]	*Organism in which protein was identified*		*Reference*	*Gene*	*Accession number*
1	Mannitol-1-phosphate dehydrogenase (M1PDH) (1.1.1.17)	Bacteria	*Escherichia coli*	Novotny et al., 1984	*mtlD*	AY523630
		Fungi	*Aspergillus niger*	Kiser and Niehaus, 1981	*mpdA*	AY081178
		Algae	*Caloglossa continua*	Iwamoto et al., 2003	—	—
2	Mannitol-1-phosphatase (M1Pase) (3.1.3.22)	Algae	*Caloglossa continua*	Iwamoto et al., 2001	—	—
3a	Mannitol 2-dehydrogenase (M2DH) (1.1.1.67)	Bacteria	*Rhodobacter sphaeroides*	Schneider and Giffhorn, 1989	*mtlK*	AF018073
		Fungi	*Absidia glauca*	Ueng et al., 1976	—	—
3b	Mannitol 2-dehydrogenase (NADP+) (M2DH) (1.1.1.138)	Bacteria	*Gluconobacter suboxydans*	Adachi et al., 1999	—	—
		Fungi	*Agaricus bisporus*	Ruffner et al., 1977	*mtdH*	AF053764
4	Hexokinase (2.7.1.1)	Bacteria	—		*hxk*	AJ510140
		Fungi	*Saccharomyces cerevisiae*	Jacob et al., 1991	*hxk1*	M14410
		Plant	—		*hxk1*	AF118132
	Sugar-phosphatase (3.1.3.23)	Bacteria	*Escherichia coli*	Choy and Lee, 1983		
		Fungi	*Saccharomyces cerevisiae*	Choy and Lee, 1983		
6	Mannose-6-phosphate isomerase (M6PI) (5.3.1.8)	Bacteria	*Escherichia coli*	Froman et al., 1989	*manA*	M15380
		Fungi	*Candida albicans*	Tolley et al., 1994	*pmi1*	X82024
7	Mannose-6-phosphate reductase (M6PR) (1.1.1.224)	Plant	*Apium graveolens*	Loescher et al., 1992	*m6pr*	U83687
8	Mannitol 1-dehydrogenase (M1DH) (1.1.1.255)	Plant	*Apium graveolens*	Stoop et al., 1998	*mtd*	U24561

[a] Number corresponds to the enzyme number in Figure 1.

ated from fructose-6-P by sugar phosphatase, and then mannitol is synthesized by the direct reduction of fructose by M2DH, as shown in Fig. 1, B (a) (Jennings, 1984; Kulkarni, 1990). The accumulated mannitol is degraded via the same pathway in the opposite direction to produce fructose-6-P by M2DH and hexokinase (Fig. 1, B (b)). Interestingly, *D. salina* also possesses an alternative pathway called a mannitol cycle (Fig. 1, B (b)), wherein mannitol is synthesized from fructose-6-P via mannitol-1-P by M1PDH and M1Pase; when degraded, the accumulated mannitol is decomposed to fructose-6-P via fructose by M2DH and hexokinase (Hult and Gatenbeck, 1978). This pathway was found in various Fungi Imperfecti, such as *Alternaria alternata* and *Aspergillus niger*, but not in phycomycetes, ascomycetes, or basidiomycetes (Hult et al., 1980; Jennings, 1984).

Mannitol Metabolism in Higher Plants

The differences in mannitol metabolism between higher plants and other organisms include the spatial separation of biosynthesis and catabolism and the involvement of mannose-6-P and mannose in the respective processes. Biosynthesis proceeds in leaves (source tissue) by sequential catalysis with phosphomannose isomerase, mannose-6-P reductase, and M1Pase (Everard et al., 1993; Stoop and Pharr, 1994; Stoop et al., 1996; Fig. 1, C, left). The mannitol is then transported to roots and very young leaves (sink tissues), where it is stored or catabolized to produce storage compounds and carbon skeletons for growth by catalysis with M1DH, hexokinase, and phosphomannose isomerase (Fig. 1, C, right).

In higher plants mannitol metabolism is regulated by the de novo biosynthesis of key enzymes, such as mannose-6-P reductase in source tissue and M1DH in sink tissue, under gene expression during development of the tissues (Everard et al., 1993; Stoop and Pharr, 1994; Stoop et al., 1996). In addition to such developmental control, mannitol metabolism is controlled by the environmental and regulatory factors governing the activity of key enzymes. Mannose-6-P reductase, which is activated by NaCl, is controlled at an activity level by the availability of NADPH that is controlled by nonreversible glyceraldehyde-3-P dehydrogenase (GAPDH; Everard et al., 1994). Moreover, the GAPDH is controlled by changes in the messenger RNA level in response to high salt stress (Gao and Loescher, 2000). In contrast, M1DH is suppressed under high-salt stress (Stoop and Pharr, 1994) because reduced gene expression decreases the amount of enzyme protein (Williamson et al., 1995). In addition to the salinity effect, the expression of M1DH is repressed by hexose sugars (Prata et al., 1997). When mannitol utilization is repressed, plants can accumulate large amounts of mannitol under normal conditions and store it under salt stress (Stoop et al., 1996; Williamson et al., 2002). In addition to mannitol-metabolizing enzymes, sugar transporters may play an important role in regulating mannitol utilization in celery (Noiraud et al., 2000, 2001).

Mannitol Metabolism in Algae

Mannitol metabolism in marine photosynthetic organisms is poorly understood in comparison to bacteria, fungi, and higher plants, regardless of the physiologic significance. In algal evolution the occurrence of mannitol metabolism is still not clear. There are no reports on mannitol metabolism activity in some algal groups, including chryptophyceans and xanthophyceans. Kremer (1976) and Bieleski (1982) suggested that the evidence for mannitol production should be reevaluated, especially for studies of rhodophycean, chrysophycean, and bacillariophycean algae published before the early 1970s, because mannitol may have been misidentified in chemical analyses, or other mannitol-producing organisms may have contaminated samples. However, it is certain that the phaeophycean algae *Eisenia*, *Dictyota*, and *Spatoglossum* (Yamaguchi et al., 1966, 1969; Ikawa et al., 1972), the prasinophycean alga *Platymonas* (Richter and Kirst, 1987), and the rhodophycean alga *Caloglossa* (Karsten et al., 1997b) can synthesize mannitol, because high activity levels of mannitol-producing enzymes, such as M1PDH and M1Pase, have been detected in these algae. In brown and prasinophycean algae, mannitol is the main product of photosynthesis (Carigie et al., 1966; Yamaguchi et al., 1966). In particular, free mannitol in brown algae composed up to 30% of the dry weight, although its levels varied among species (Reed et al., 1985). The metabolic pathway for mannitol in algae is essentially the same as the fungal cycle (Fig. 1, B (b)): mannitol synthesis is mediated by M1PDH and M1Pase, and catabolism is catalyzed by M2DH and hexokinase to produce fructose-6-P via fructose (Ikawa et al., 1972; Richter and Kirst, 1987; Karsten et al., 1997b). The difference in the algal cycle is the requirement for NAD in the oxidation of mannitol by M2DH, whereas the fungal cycle utilizes NADP (Hult and Gatenbeck, 1978).

The red alga *Caloglossa* is a eulittoral macrophyte that grows epiphytically on bank sides or reed stems in estuaries. The alga is subjected to osmotic stress by evaporation, rainfall, and the large changes in salinity during low and high tides. Therefore the alga needs to adjust its osmotic pressure to prevent osmotic damage (Karsten et al., 1992, 1994; West et al., 1992). It acclimates to osmotic changes by inducing mechanisms for controlling intracellular concentrations of mannitol. The biosynthesis of mannitol in *Caloglossa* is unusual for a red alga, since red macrophytes usually synthesize heteroside floridoside, isofloridoside (Bangiales), or digeneaside (Ceramiales) as the major products of photosynthesis (Evans et al., 1973; Kremer and Kirst, 1982; Karsten et al., 1999). Physiologic data have been compiled for *Caloglossa* species on how concentrations of intracellular salt ions and mannitol change in response to salinity changes (Mostaert et al., 1995a, 1995b). Hence this genus could serve as a model organism for investigating how algae acclimate to salinity changes by regulating mannitol metabolism. Karsten and West (1993) reported that mannitol accumulated significantly under hypertonic conditions in 6 species of *Caloglossa*. Changes in mannitol concentration in *C. leprieurii* depended on salinity (Mostaert et al., 1995a) and required 8 hours to accumulate a detectable amount and 24 hours to attain a new steady state after exposure to hypersaline conditions. In contrast, the mannitol concentration decreased markedly within 1 hour when the alga was exposed to hyposaline conditions (Mostaert et al., 1995b).

Significant progress has been made in clarifying the regulation of mannitol biosynthesis in *Caloglossa*. Two enzymes in mannitol biosynthesis, M1PDH and M1Pase, have been purified and characterized in *C. continua* by introducing a 2-phase partitioning system with polyethylenglycol and ammonium sulfate (Iwamoto et al., 2001, 2003; Iwamoto and Shiraiwa, 2005). M1PDH reacted only with fructose-6-P and NADH in the fructose-6-P-reducing reaction and only with mannitol-1-P and NAD in the mannitol-1-P-oxidizing reaction. The substrate specificity of M1Pase was also high, because only trace activity was detected with polyol phosphates, such as sorbitol-1-P and fructose-6-P. These results indicate that these enzymes are specific for mannitol metabolism. M1Pase was partially inhibited by a high concentration of its mannitol product. Therefore, mannitol biosynthesis can be controlled by feedback regulation. Metal ions may also be involved in the regulatory mechanism, because the M1Pase activity was increased about 10-fold by 1 mM Mg^{2+}, but inactivated by Ca^{2+}. M1PDH was not affected by the ion. The fructose-6-P-reducing activity of M1PDH, which catalyzes the conversion of fructose-6-P to mannitol-1-P, was increased about 3 times by the addition of 150 mM NaCl. However, the mannitol-1-P-oxidizing activity of M1PDH, a reverse reaction of fructose-6-P reduction, was not affected by NaCl (Fig. 2), while M1Pase was inhibited 60% by a seawater level (500 mM) of NaCl.

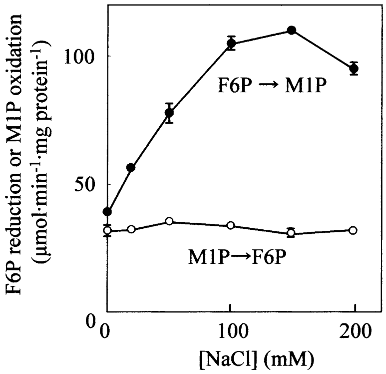

Fig. 2. Effects of NaCl concentration on activity of M1PDH purified from red alga *Caloglossa continua*. Closed circle, fructose-6-P reduction. Open circle, mannitol-1-P oxidation (modified from Iwamoto et al., 2003). Error bars indicate the minimum and maximum values in the duplicated examination.

The enzyme-level regulation of mannitol biosynthesis by salt is well supported. Intracellular concentrations of Cl^-, Na^+, and K^+ were increased from 38 mM, 8 mM, and 94 mM to 177 mM, 17 mM, and 156 mM, respectively, when freshwater-acclimated *C. leprieurii* was transferred to seawater (Mostaert et al., 1995b). The mechanism by which M1PDH is activated by NaCl is complex and involves 3 parameters: NaCl concentration, fructose-6-P concentration, and optimal pH. As shown in Fig. 3, salt concentration greatly changed the dependence of fructose-6-P reduction on fructose-6-P concentration. In the absence of NaCl, fructose-6-P-reducing activity was saturated at 0.5 mM fructose-6-P and strongly inhibited by the substrate at levels above 0.5 mM. In contrast, the addition of 200 mM NaCl elevated maximal activity from 0.5 to 2.5 mM and simultaneously eliminated the substrate inhibition. Thus a high NaCl concentration greatly decreases the affinity of M1PDH for fructose-6-P. This change in activity and kinetic parameters may result partly from changes in the optimal pH. The addition of 200 mM NaCl to a 5 mM fructose-6-P concentration shifted the optimal pH for fructose-6-P reduction to about 7 from less than 6.0. Interestingly, adding NaCl to a 0.5 mM fructose-6-P concentration did not change the optimal pH. As M1PDH is a cytoplasmic enzyme, a shift in the optimal pH from the neutral range would inactivate the enzyme in vivo (Karsten et al., 1997b).

Figure 4 summarizes the metabolic pathways of mannitol in the red alga *C. continua*. The principal pathway is almost identical to that in fungi, except that NAD-dependent M2DH (Table 1, 3a) is in-

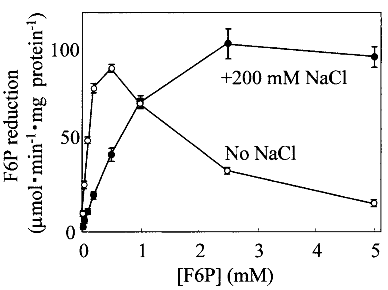

Fig. 3. Kinetic analysis of relation between fructose-6-P-reducing activity and fructose-6-P concentration in M1PDH purified from red alga *Caloglossa continua*. Open circle, assayed without NaCl. Closed circle, assayed with 200 mM NaCl (modified from Iwamoto et al., 2003). Error bars indicate the minimum and maximum values in the duplicated examination.

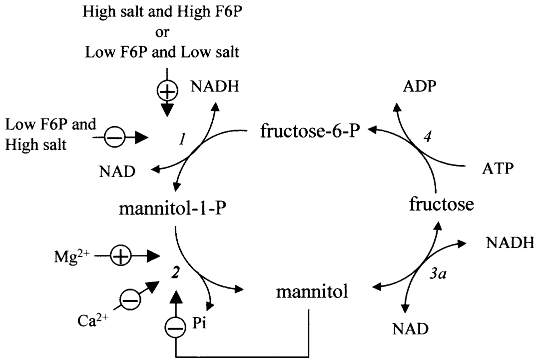

Fig. 4. Regulatory mechanism of mannitol metabolism in red alga *Caloglossa* (modified from Iwamoto et al., 2004). 1, M1PDH; 2, M1Pase; 3a, M2DH; 4, hexokinase. Circled minus and plus symbols, down- and upregulation of the reaction.

volved in *C. continua*. Mannitol biosynthesis may be directly regulated at the enzyme level by metal ions such as Mg^{2+}, product feedback control, and salt. This system would allow rapid response to environmental salinity changes without the time lag required for the de novo synthesis of enzymes. Other regulatory mechanisms activating M1PDH and M1Pase may also be involved. The involvement of the fructose-6-P concentration as an activating mechanism of M1PDH indicates that mannitol synthesis is also regulated by the fructose-6-P supply system. NADH supply may also control the pathway, as nonreversible GAPDH regulates mannitol biosynthesis in higher plants (Gao and Loescher, 2000).

When salt stress is released, mannitol synthesis is disrupted by the inactivation of M1PDH and M1Pase, in a reverse manner of their activation. Simultaneously, M2DH and hexokinase begin to degrade mannitol (Karsten et al., 1997b; Fig. 4). Considering the importance of mannitol degradation enzymes in the regulation of mannitol metabolism in higher plants (Stoop et al., 1996), they could be expected to be an important factor in elucidating mannitol metabolism in mannitol-producing algae. However, the characterization of M2DH in algae is poor. Therefore understanding mannitol metabolism in algae requires extensive investigation of the properties and regulation of this enzyme.

Application to the Production of Salt-Tolerant Transgenic Plants

Mannitol biosynthesis is one of the most extensively tested targets for improving salt tolerance in horticultural crops by genetic engineering. According to Flowers (2004) salt tolerance was enhanced in 6 plant transformation experiments performed between 1993 and 2003 that introduced *mtlD*, the gene encoding M1PDH. The plants used were tobacco (Tarczynski et al., 1993; Karakas et al., 1997), *Arabidopsis* (Thomas et al., 1995), wheat (Abebe et al., 2003), and rice (Lee et al., 2000; Wang et al., 2000). However, the transgenic plants showed little improvement in their salinity tolerance. One possible reason is that the transgenic plants accumulated relatively low levels of mannitol compared with mannitol-accumulating salt-tolerant plants (Reed et al., 1985; Tarczynski et al., 1992; Karsten and West, 1993; Stoop and Pharr, 1994). Carbon and energy metabolism may also have been disrupted by the overexpression of *mtlD* (Hare et al., 1998; Abebe et al., 2003).

Mannitol-metabolizing enzymes in *C. continua* exhibit unique properties in regulating enzyme activation compared with bacterial M1PDH (Watanabe et al., 2003). Since the *Caloglossa* enzyme is autoregulated by the balance of the intracellular concentration of NaCl and fructose-6-P, the introduction of *Caloglossa* M1PDH instead of *E. coli* M1PDH could be expected to improve the salt tolerance of transgenic plants without disturbing cellular metabolism. The introduction of *Caloglossa* M1Pase in addition to M1PDH may further benefit the plants. The mannitol-accumulating transgenic land plants produced so far do not possess M1Pase; instead, the final step of mannitol biosynthesis is catalyzed by a substrate non specific phosphatase (Tarczynski et al., 1992; Thomas et al., 1995). The level of mannitol accumulation should be higher in transgenic plants possessing M1PDH and M1Pase, as *Caloglossa* M1Pase exhibits high specificity and affinity to mannitol-1-P (Iwamoto et al., 2001). Introducing M1Pase should also confer the advantage of directing the subcellular localization of mannitol by expression in the cytosol, similar to other mannitol-accumulating photosynthetic organisms (Rumpho et al., 1983; Karsten et al., 1997b). Furthermore, introducing the enzyme may avoid over accumulation of mannitol, as *Caloglossa* M1Pase activity is subject to product feedback regulation by mannitol. Therefore introducing both *Caloglossa* M1PDH and M1Pase may solve the problems of carbon and energy metabolism disruption, over accumulation of mannitol, and accumulation under nonstress conditions (Hare et al., 1998; Abebe et al., 2003).

Acknowledgments

We thanks Dr. John Beardall for his kind and critical reading of this manuscript. The study on algal mannitol metabolism was supported in part by the Salt Science Foundation (Tokyo, Japan) in 2000 (funding no. 0020) and 2001 (funding no. 0124).

References

1. Abebe T, Guenzi AC, Martin B, Cushman JC (2003) Tolerance of mannitol-accumulating transgenic wheat to water stress and salinity. Plant Physiol 131, 1748–1755
2. Adachi O, Toyama H, Matsushita K (1999) Crystalline NADP-dependent D-mannitol dehydrogenase from *Gluconobacter suboxydans*. Biosci Biotechnol Biochem 63, 402–407
3. Allaway AE, Jennings DH (1970) The influence of cations on glucose transport and metabolism by, and the loss of sugar alcohols from, the fungus *Dendryphiella salina*. New Phytol 69, 581–593
4. Apostol I, Heinstein PF, Low PS (1989) Rapid stimulation of an oxidative burst during elicitation of cultured plant cells: role in defense and signal transduction. Plant Physiol 90, 109–116
5. Armstrong RA, Smith SN (1998) Does radial growth of the lichen *Parmelia conspersa* depend exclusively on growth processes at the lobe tip? Environ Exp Bot 39, 263–269
6. Ben-Amotz A, Avron M (1983) Accumulation of metabolites by halotolerant algae and its industrial potential. Annu Rev Microbiol 37, 95–119
7. Bieleski RL (1982) Sugar alcohols. In: *Encyclopedia of Plant Physiology. New Series 13-A. Plant Carbohydrates*, Loewus FA, Tanner W, eds. (Berlin, Germany: Springer-Verlag) pp 158–192
8. Carigie JS, McLachlan J, Majak W (1966) Photosynthesis in algae, II: green algae with special reference to *Dunaliella* spp. and *Tetraselmis* spp. Can J Bot 44, 1247–1254
9. Choy FYM, Lee YP (1983) Survey, purification, properties of sugar phosphate phosphohydrolase among microorganisms. Can J Biochem Cell Biol 61, 1292–1303
10. Davison IR, Reed RH (1985) The physiological significance of mannitol accumulation in brown algae: the role of mannitol as a compatible cytoplasmic solute. Phycologia 24, 449–457
11. Delavault P, Simier P, Thoiron S, Véronési C, Fer A, Thalouarn P (2002) Isolation of mannose 6-phosphate reductase cDNA, changes in enzyme activity and mannitol content in broomrape (*Orobanche ramosa*) parasitic on tomato roots. Physiol Plantarum 115, 48–55
12. Evans LV, Callow JA, Callow ME (1973) Structural and physiological studies on the parasitic red alga *Holmsella*. New Phytol 72, 393–402
13. Everard JD, Franceschi VR, Loescher WH (1993) Mannose-6-phosphate reductase, a key enzyme in photoassimilate partitioning, is abundant and located in the cytosol of photosynthetically active cells of celery (*Apium graveolens* L.) source leaves. Plant Physiol 102, 345–356
14. Everard JD, Gucci R, Kann SC, Flore JA, Loescher WH (1994) Gas exchange and carbon partitioning in the leaves of celery (*Apium graveolens* L.) at various levels of root zone salinity. Plant Physiol 106, 281–292
15. Flowers TJ (2004) Improving crop salt tolerance. J Exp Bot 55, 307–319
16. Gao Z, Loescher WH (2000) NADPH supply and mannitol biosynthesis: characterization, cloning, and regulation of the non-reversible glyceraldehyde-3-phosphate dehydrogenase in celery leaves. Plant Physiol 124, 321–330
17. Froman BE, Tait RC, Gottlieb LD (1989) Isolation and characterization of the phosphoglucose isomerase gene from *Escherichia coli*. Mol Gen Genet 217, 126–131
18. Hare PD, Cress WA, Van Staden J (1998) Dissecting the roles of osmolyte accumulation during stress. Plant Cell Environ 21, 535–553
19. Hellebust JA (1976) Effect of salinity on photosynthesis and mannitol synthesis in the green flagellate *Platymonas suecica*. Can J Bot 54, 1735–1741
20. Hult K, Gatenbeck S (1978) Production of NADPH in the mannitol cycle and its relation to polyketide formation in *Alternaria alternata*. Eur J Biochem 88, 607–612
21. Hult K, Veide A, Gatenbeck S (1980) The distribution of the NADPH regenerating mannitol cycle among fungal species. Arch Microbiol 128, 253–255
22. Ikawa T, Watanabe T, Nisizawa K (1972) Enzymes involved in the last steps of the biosynthesis of mannitol in brown algae. Plant Cell Physiol 13, 1017–1029
23. Iwamoto K, Shiraiwa Y (2005) Technical improvement in the purification of enzymes from red algae using an aqueous two-phase partitioning system. Phycol Res (in press)
24. Iwamoto K, Kawanobe H, Shiraiwa Y, Ikawa T (2001) Purification and characterization of mannitol-l-phosphatase in the red alga *Caloglossa continua* (Ceramiales, Rhodophyta). Mar Biotechnol 3, 493–500
25. Iwamoto K, Kawanobe H, Ikawa T, Shiraiwa Y (2003) Characterization of salt-regulated mannitol-1-phosphate dehydrogenase in the red alga *Caloglossa continua*. Plant Physiol 133, 893–900

26. Iwamoto K, Kawanobe H, Ikawa T, Shiraiwa Y (2004) Regulatory mechanism of mannitol biosynthesis in the red alga *Caloglossa continua* (Ceramiales, Rhodophyta). Jpn J Phycol 52(suppl), 101–105
27. Jacob L, Beecken V, Bartunik LJ (1991) Purification and crystallization of yeast hexokinase isoenzymes: characterization of different forms by chromatofocusing. J Chromatogr 587, 85–92
28. Jennings DH (1984) Polyol metabolism in fungi. Adv Microb Physiol 25, 149–193
29. Jennings DB, Ehrenshaft M, Pharr DM, Williamson JD (1998) Roles for mannitol and mannitol dehydrogenase in active oxygen-mediated plant defense. Proc Natl Acad Sci USA 95, 15129–15133
30. Jennings DB, Daub ME, Pharr DM, Williamson JD (2002) Constitutive expression of a celery mannitol dehydrogenase in tobacco enhances resistance to the mannitol-secreting fungal pathogen *Alternaria alternata*. Plant J 32, 41–49
31. Joosten MHAJ, Hendrickx LJM, de Wit PJGM (1990) Carbohydrate composition of apoplastic fluids isolated from tomato leaves inoculated with virulent or avirulent races of *Cladosporium fulvum* (syn. *Fulvia fulva*). Neth J Plant Path 96, 103–112
32. Karakas B, Ozias-Akins P, Stushnoff C, Suefferheld M, Rieger M (1997) Salinity and drought tolerance of mannitol-accumulating transgenic tobacco. Plant Cell Environ 20, 609–616
33. Karsten U, West JA (1993) Ecophysiological studies on six species of the mangrove red algal genus *Caloglossa*. Aust J Plant Physiol 20, 729–739
34. Karsten U, West JA, Mostaert AS, King. R.J, Barrow KD, Kirst GO (1992) Mannitol in the red algal genus *Caloglossa* (Harvey). J Agardh J Plant Physiol 140, 292–297
35. Karsten U, Barrow KD, Mostaert AS, King RJ, West JA (1994) ^{13}C- and ^{1}H-NMR studies on digeneaside in the red alga *Caloglossa leprieurii:* a re-evaluation of its osmotic significance. Plant Physiol Biochem 32, 669–676
36. Karsten U, Barrow KD, West JA, King RJ (1997a) Mannitol metabolism in the intertidal mangrove red alga *Caloglossa leprieurii*: salinity effects on enzymatic activity. Phycologia 36, 150–156
37. Karsten U, Barrow KD, Nixdorf O, West JA, King RJ (1997b) Characterization of mannitol metabolism in the mangrove red alga *Caloglossa leprieurii* (Montagne). J Agardh Planta 201, 173–178
38. Karsten U, West JA, Zuccarello GC, Nixdorf O, Barrow KD, King RJ (1999) Low molecular weight carbohydrate patterns in the Bangiophyceae (Rhodophyta). J Phycol 35, 967–976
39. Keller T, Damude HG, Werner D, Doerner P, Dixon RA, Lamb C (1998) A plant homolog of the neutrophil NADPH oxidase gp91^{phox} subunit gene encodes a plasma membrane protein with Ca^{2+} binding motifs. Plant Cell 10, 255–266
40. Kiser RC, Niehaus WG Jr (1981) Purification and kinetic characterization of mannitol-1-phosphate dehydrogenase from *Aspergillus niger*. Arch Biochem Biophys 211, 613–621
41. Kremer BP, Willenbrink J (1972) CO_2-Fixierung und Stofftransport in benthischen marinen Algen, I: Zur Kinetik der $^{14}CO_2$-Assimilation bei *Laminaria saccharina*. Planta 103, 55–64
42. Kremer BP (1976) Mannitol in the rhodophyceae—a reappraisal. Phytochemistry 15, 1135–1138
43. Kremer BP, Kirst GO (1982) Biosynthesis of photosynthates and taxonomy of algae. Z Naturforsch 37, 761–771
44. Kulkarni RK (1990) Mannitol metabolism in *Lentinus edodes*, the shiitake mushroom. Appl Environ Microbiol 56, 250–253
45. Lee EA, Kim JD, Cha YK, Woo DH, Han IS (2000) Transiently expressed salt-stress protection of rice by transfer of a bacterial gene, mtlD. J Microbiol Biotechnol 10, 415–418
46. Loescher WH, Tyson RH, Everard JD, Redgewell J, Bieleski RL (1992) Mannitol synthesis in higher plants: evidence for the role and characterization of a NADPH-dependent mannose 6-phosphate reductase. Plant Physiol 98, 1396–1402
47. Mostaert AS, Karsten U, King RJ (1995a) Physiological responses of *Caloglossa leprieurii* (Ceramiales, Rhodophyta) to salinity stress. Phycol Res 43, 215–222
48. Mostaert AS, Karsten U, King RJ (1995b) Inorganic ions and mannitol in the red alga *Caloglossa leprieurii* (Ceramiales, Rhodophyta): response to salinity change. Phycologia 34, 501–507
49. Munda I (1964) The quantity and chemical composition of *Ascophyllum nodosum* (L) Le Jol along the coast between the rivers Ölfusá and Thjorsá (Southern Iceland). Bot Mar 7, 76–89
50. Noiraud N, Delrot S, Lemoine R (2000) The sucrose transporter of celery: identification and expression during salt stress. Plant Physiol 122, 1447–1455
51. Noiraud N, Maurousset L, Lemoine R (2001) Identification of a mannitol transporter, AgMaT1, in celery phloem. Plant Cell 13, 695–705
52. Novotny MJ, Reizer J, Esch F, Saier MH Jr (1984) Purification and properties of D-mannitol-1-phosphate dehydrogenase and D-glucitol-6-phosphate dehydrogenase from *Escherichia coli*. J Bacteriol 159, 986–990
53. Prata RTN, Williamson JD, Conkling MA, Pharr DM (1997) Sugar repression of mannitol dehydrogenase activity in celery cells. Plant Physiol 114, 307–314
54. Reed RH, Davison IR, Chudek JA, Foster R (1985) The osmotic role of mannitol in the Phaeophyta: an appraisal. Phycologia 24, 35–47
55. Richter DFE, Kirst GO (1987) D-mannitol dehydrogenase and D-mannitol-1-phosphate dehydrogenase in *Platymonas subcordiformis*: some characteristics and their role in osmotic adaptation. Planta 170, 528–534
56. Robert S, Simier P, Fer A (1999) Purification and characterization of mannose 6-phosphate reductase, a potential target for the control of *Striga hermonthica* and *Orobanche ramosa*. Aust J Plant Physiol 26, 233–237

57. Ruffner HS, Rast D, Tobler H, Karesch H (1977) Purification and properties of mannitol dehydrogenase form *Agaricus bisporus* sporocarps. Phytochemistry 17, 865–868
58. Rumpho ME, Edwards GE, Loescher WH (1983) A pathway for photosynthetic carbon flow to mannitol in celery leaves: activity and localization of key enzymes. Plant Physiol 73, 869–873
59. Schmatz DM, Baginsky WF, Turner MJ (1989) Evidence for and characterization of mannitol cycle in *Eimeria tenella*. Mol Biochem Parasit 32, 263–270
60. Schmitz K, Srivastava LM (1975) On the fine structure of sieve tubes and the physiology of assimilate transport in *Alaria marginata*. Can J Bot 53, 861–876
61. Schneider KH, Giffhorn F (1989) Purification and properties of a polyol dehydrogenase from the phototrophic bacterium *Rhodobacter sphaeroides*. Eur J Biochem 184, 15–19
62. Shen B, Jensen RG, Bohnert HJ (1997a) Increased resistance to oxidative stress in transgenic plants by targeting mannitol biosynthesis to chloroplasts. Plant Physiol 113, 1177–1183
63. Shen B, Jensen RG, Bohnert HJ (1997b) Mannitol protects against oxidation by hydroxyl radicals. Plant Physiol 115, 527–532
64. Simier P, Robert S, Fer A (1998) Mannitol metabolism in darkness in the leaves of the hemiparasitic angiosperm, *Thesium humile*. Plant Physiol Biochem 36, 237–245
65. Smirnoff N, Cumbes Q (1989) Hydroxyl radical scavenging activity of compatible solutes. Phytochemistry 28, 1057–1060
66. Stoop JMH, Pharr DM (1994) Mannitol metabolism in celery stressed by excess macronutrients. Plant Physiol 106, 503–511
67. Stoop JMH, Williamson JD, Conkling MA, MacKay JJ, Pharr DM (1998) Characterization of NAD-dependent mannitol dehydrogenase from celery as affected by ions, chelators, reducing agents and metabolites. Plant Sci 131, 43–51
68. Stoop JMH, Williamson JD, Pharr DM (1996) Mannitol metabolism in plants: a method for coping with stress. Trends Plant Sci 1, 139–144
69. Stoop JMH, Mooibroek H (1998) Cloning and characterization of NADP-mannitol dehydrogenase cDNA from the button mushroom, *Agaricus bisporus*, its expression in response to NaCl stress. Appl Environ Microbiol 64, 4689–4696
70. Tandon SK, Singh S, Prasad S, Khandekar K, Dwivedi VK, Chatterjee A, Mathur N (2003) Reversal of cadmium induced oxidative stress by chelating agent, antioxidant or their combination in rat. Toxicol Lett 145, 211–217
71. Tarczynski MC, Jensen RG, Bohnert HJ (1992) Expression of a bacterial mtlD gene in transgenic tobacco leads to production and accumulation of mannitol. Proc Natl Acad Sci USA 89, 2600–2604
72. Tarczynski MC, Jensen RG, Bohnert HJ (1993) Stress protection of transgenic tobacco by production of the osmolyte mannitol. Science 259, 508–510
73. Thomas JC, Sepahi M, Arendall B, Bohnert HJ (1995) Enhancement of seed germination in high salinity by engineering mannitol expression in *Arabidopsis thaliana*. Plant Cell Environ 18, 801–806
74. Tolley S, Davies G, Hubbard RE, Smith DJ, Proudfoot AEI, Payton MA, Cleasby A, Wonacott A, Wells TNC (1994) Crystallization and preliminary X-ray analysis of *Candida albicans* phosphomannose isomerase. J Mol Biol 237, 349–350
75. Ueng ST, Hartanowicz P, Lewandoski C, Keller J, Holick M, McGuinness ET (1976) D-Mannitol dehydrogenase from *Absidia glauca*: purification, metabolic role, and subunit interactions. Biochemistry 15, 1743–1749
76. Wang HZ, Huang DN, Lu RF, Liu JJ, Qian Q, Peng XX (2000) Salt tolerance of transgenic rice (*Oryza sativa* L.) with *mtlD* gene and *gutD* gene. Chinese Sci Bull 45, 1685–1690
77. Watanabe S, Hamano M, Kakeshita H, Bunai K, Tojo S, Yamaguchi H, Fujita Y, Wong SL, Yamane K (2003) Mannitol-1-phosphate dehydrogenase (MtlD) is required for mannitol and glucitol assimilation in *Bacillus subtilis*: possible cooperation of *mtl* and *gut* operons. J Bacteriol 185, 4816–4824
78. West JA, Zuccarello GC, Pedroche FF, Karsten U (1992) Marine red algae of the mangroves in Pacific Mexico and their polyol content. Bot Mar 35, 567–572
79. Williamson JD, Stoop JMH, Massel MO, Conkling MA, Pharr DM (1995) Sequence analysis of a mannitol dehydrogenase cDNA from plants reveals a function for the pathogenesis-related protein ELI3. Proc Natl Acad Sci USA 92, 7148–7152
80. Williamson JD, Jennings DB, Guo WW, Pharr DM, Ehrenshaft M (2002) Sugar alcohols, salt stress, and fungal resistance: polyols — multifunctional plant protection? J Am Soc Hortic Sci 127, 467–473
81. Wisselink HW, Weusthuis RA, Eggink G, Hugenholtz J, Grobben GJ (2002) Mannitol production by lactic acid bacteria: a review. Int Dairy J 12, 151–161
82. Yamaguchi T, Ikawa T, Nisizawa K (1966) Incorporation of radioactive carbon from $H^{14}CO_3^-$ into sugar constituents by a brown alga, *Eisenia bicyclis*, during photosynthesis and its fate in the dark. Plant Cell Physiol 7, 217–229
83. Yamaguchi T, Ikawa T, Nisizawa K (1969) Pathway of mannitol formation during photosynthesis in brown algae. Plant Cell Physiol 10, 425–440
84. Yancey PH, Clark ME, Hand SC, Bowlus RD, Somero GN (1982) Living with water stress: evolution of osmolyte systems. Science 217, 1214–1222
85. Yu TH, Bai J, Hu K, Wang ZB (2003) The effect of free radical scavenger and antioxidant on the increase in intracellular adriamycin accumulation induced by ultrasound. Ultrason Sonochem 10, 33–35