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REVIEW ARTICLE

Agro-Industrial Uses of Glycinebetaine

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In addition to sugar, several different compounds are presently separated from beet molasses and juices. Nowadays, some of these products have proved to be economically even more important to beet sugar factories than the original product, sugar. One of these compounds is glycinebetaine (N, N', N"-trimethylglycine, GB), an amino acid derivative accumulated in many microbes and plant species grown under stress, but also in humans. Especially halophytes belonging to families Amaranthaceae, Asteraceae, Chenopodiaceae, Convolvulaceae, Graminaceae, Malvaceae, Poaceae and Portulaceae synthesize and accumulate GB. GB is assumed to have several adaptive effects on drought and salt stressed plants according to studies mostly based on research work established with cell cultures, bacteria, or isolated chloroplasts. The known role of GB is to maintain water content in animal and plant cells by lowering solute potential under osmotic stress, i.e. to act in osmotic adjustment. This has offered a wide field for use of GB in industry and agriculture for various purposes.

KEYWORDS : Glycinebetaine, crop production, sugarbeet molasses

At a certain point of sugar processing no more sugar can be crystallized from molasses which contain virtually all of the original GB. Therefore, molasses is used as a raw material for the production of GB. The extraction is based on chromatographic separation process. This process is carried out in large columns filled with separation resin (Fig. 1). Water is used to elute the molasses through the column system. At the outlet points of the column system, the different compounds, like GB, can be collected as almost pure solutions. These are concentrated and crystallized into pure natural, anhydrous GB. The other source for industrially purified non-synthetic GB is marine brown algae *Ascophyllum nodosum* (L.) Le Jol. (Whapman *et al.*, 1993; Blunden *et al.*, 1997). Synthetic GB is also available from Sigma chemical company.

Sugar beet (*Beta vulgaris* L. v. *altissima*) is a crop species grown in northern latitudes that accumulates GB also when no stresses occur. The GB concentration in sugar beet is especially dependent on the variety. Old varieties usually contain more GB than the new ones, as breeding has been targeted for better extraction of sugars through reducing compounds such as GB. Water shortages and low plant canopy density during the growing period result in increased rate of GB accumulation in sugar beet (Beiß, 1994). Furthermore, a high positive correlation exists between increased potassium fertilizer application rate and GB concentration in sugar beet root. Moreover, GB concentration is known to increase towards the end of the growing season being highest in the upper parts of the sugar beet storage root (Beringer *et al.*, 1986; Beiß, 1994).

In Chenopods including sugar beet, GB is synthesized (Fig. 2) by a two step oxidation pathway of choline in the chloroplast (McCue and Hanson, 1990). The first step is catalysed by choline mono-oxygenase (CMO) and results in hydrate form of betaine aldehyde. The next step is synthesis of betaine which is catalyzed by betaine aldehyde dehydrogenase (BADH) (Broquisse *et al.*, 1989). The choline for GB synthesis is formed from serine which in turn is an intermediate product of photo-oxidative carbon metabolism and photorespiration (Papageorgiou *et al.*, 1991). It has been postulated that light is essential for the synthesis of GB as the first step requires reduced ferredoxin (Arakawa *et al.*, 1992). McCue and Hanson (1990) have suggested that the enzyme activities in GB synthesis are regulated possibly by osmotic stress. However, in

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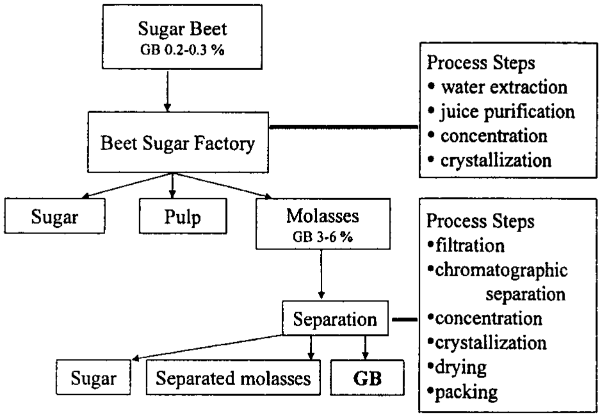

Fig. 1 : The method of GB separation in sugar processing from sugar beets.

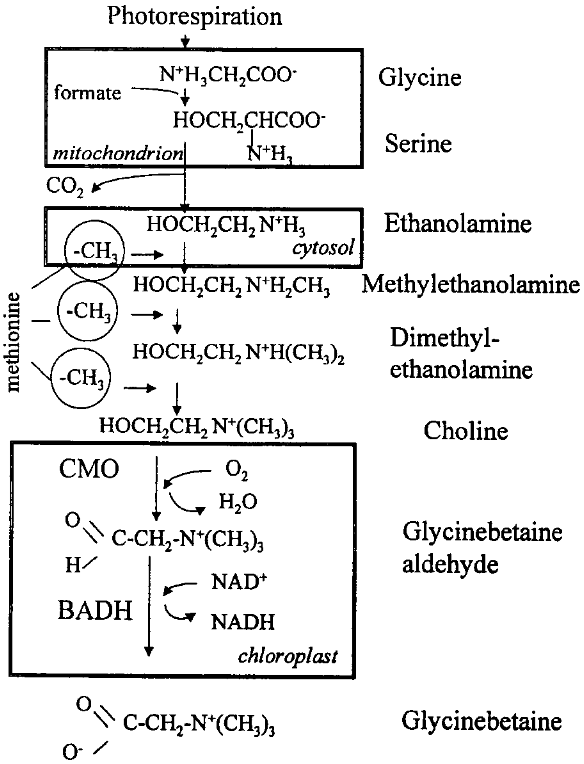

Fig. 2. The proposed pathway of GB synthesis in chenopods (including sugar beet) after Wyn Jones and Storey (1981), Hanson and Grumet (1985), Hanson et al. (1995) and Gorham (1995). CMO, choline mono-oxygenase; BADH betaine aldehyde dehydrogenase.

sugar beet the signal that causes the rise in BADH mRNA is still under investigation (Hanson *et al.*, 1995). It does not seem to be reduction in turgor, accumulation of NaCl in plant tissue nor abscisic acid (ABA) but some biochemical factor translocated from roots to shoot. The oxidation steps are physiologically irreversible and the end product, GB, metabolically quite inert in plants (Hanson and Grumet, 1985). The synthesis of GB for plants is, however, energetically expensive as it requires nitrogen, and the biosynthetic pathway is dependent on NADPH as reducing power (Gorham, 1995).

GLYCINEBETAINE IN ANIMAL PRODUCTION

In a volumetric scale, application of GB for animal nutrition is globally the largest. The use of GB in animal feed is based on its two metabolic roles in animals as a methyl donor and an osmolyte. GB molecule has three methyl groups. One of those is labile and primarily used to methylate homocysteine to methionine (Finkelstein, 1990). Two other methyl groups of GB are transferred to folate providing an alternative route for homocysteine methylation (Frontiera *et al.*, 1994). Although GB is synthesized endogenously from choline in most animal tissues it does not seem to be sufficient for the needs of methylation, especially when environmental factors such as pathogen challenge affect the need of methionine. This has been demonstrated with chicken as 40-50 % of the choline requirement and 20-25 % of the methionine requirement in diet could be replaced with GB supplementation (Frontiera *et al.*, 1994).

The osmolytic function of GB in animal metabolism is maintenance of the osmotic strength of cells and stabilization of macromolecular functions. GB has been shown to improve water retention in the epithelium of hyperosmotically stressed chicken small intestine. Actually, GB supplementation has been used for many years to relieve osmotic stress of salmonid fish when transferred from fresh water to sea water (Clarke *et al.*, 1994). Moreover, orally given GB has been reported to relieve diarrhea and dehydration in various animals as well as inhibit the invasion of coccidian (*Eimeria* sp.) parasite into gut epithelium (Ferket, 1994; Augustine and McNaughton, 1996).

The lipotropic property of GB as a methyl donor (Saunderson and Mackinlay, 1990; Barak *et al.*, 1993) has been utilized especially in the animal industry in order to reduce carcass fat and prevent excess accumulation of fat into the liver.

GLYCINEBETAINE IN CROP PRODUCTION

The role of quaternary ammonium (QAC) and tertiary sulfonium (TSC) compounds as nontoxic compatible solutes has been recognized for years in studies of plant adaptation to saline or dry environments (Rhodes and Hanson, 1993). GB is the most studied of these compounds (Hanson *et al.*, 1995). The reason for that may lie in the nature of GB as it does not inhibit enzymes even at high concentrations (Papageourgiou and Murata, 1995). Therefore, it can be accumulated in the cytoplasm of plant cells to contribute to the osmotic balance between the cytoplasm and vacuole without causing any damages. This unique nature of GB has led to that that sugar beet molasses derived foliar-applied GB is widely used in crop production throughout the world for different purposes. It is used for increasing the cold resistance of e.g. potatoes in the early spring, to increase the drought and salt resistance of e.g. tomatoes and pruning and for many other purposes. It is also used to lengthen the storage life of iceberg lettuce (Hurme *et al.*, 1999). Genetic engineering of endogenous GB in crop plants has been the focus of several groups. However, exogenous applications of GB have proven so far more effective means of crop manipulation since, the genetically engineered synthesis of GB has not yet succeeded satisfactorily (Huang *et al.*, 2000; McNeil *et al.*, 2000).

Although exogenously applied GB may be metabolized by micro-organisms soon after application (Gorham, 1995), encouraging results have been obtained with foliar- (Agboma *et al.*, 1997a,b; Mäkelä *et al.*, 1996b, 1997) and root-applied (Hofinger *et al.*, 1976) GB. In spite of e.g. the risk of microbes utilizing exogenously applied GB, foliar-applied GB is readily taken up and translocated within hours to roots and developing leaves probably along with assimilation products (Mäkelä *et al.*, 1996a). It is even translocated from roots to soil and thereafter to other plants (unpublished). However, the rate of uptake and consequent GB concentration in the plant tissue seem to be not only dependent on plant organ and its age but also on crop species and environmental factors (Mäkelä *et al.*, 1996a). Co-application of surfactant, vegetable oils in particular, tends to increase uptake by GB probably due to faster rate of uptake and thus minimizing the risk of rain flushing the GB off of the leaf foliage. Moreover, the droplets formed are smaller in size and the wax layers on the leaf surface are modified by most surfactants used thus enabling faster rate of uptake (Mäkelä *et al.*, 1996a).

GB is regarded as rather inert compound (Gorham, 1995), but there are however some opposite indications. For example, alfalfa (*Medicago sativa* L.) and its rhizobia (*Rhizobium meliloti* 102 F 34) have been reported to be able to catabolise GB (Pocard *et al.*, 1991). However, it is not known which microbes existing in soil and on the leaf surface are able to catabolise GB and to what extent (Gorham, 1995). Endogenously accumulated GB has also been reported to attract, and thereby to increase the survival rate and reproduction of aphids and plant pathogens (Araya *et al.*, 1991; Zuniga, 1989). Thus, it might be possible that exogenously applied GB also attracts and increases the rate of plant pathogens and insects in treated plants and hence, decreases realization of the yield potential. However, contrasting results have also been reported. According to Whapman *et al.* (1993) there are indications of increased resistance of GB treated wheat against *Puccinia recondite tritici* Eriks. & Henn. Also, tomato cultivars treated with GB showed increased resistance against *Meloidogyne javanica* and *M. incognita* nematodes (Wu *et al.*, 1997).

The earliest experiments with exogenous GB in plant production were conducted by Hofinger *et al.* (1976) and by Itai and Paleg (1982). According to Hofinger *et al.* (1976) GB behaved as a strong inhibitor of root growth when applied at higher concentrations than 50 mM on the growth medium of lentil, although it stimulated the growth of roots when applied at concentrations between 1 mM and 50 mM. Itai and Paleg (1982) reported increased growth of drought-recovering barley (*Hordeum vulgare* L.) when GB (25 mM) was applied on the leaf foliage of stressed plants towards the end of the stress period.

Field experiments conducted in drought-prone environments and in saline soils have indicated that the crop stability and yield was often increased due to foliar applications of GB when sprayed prior to stress. For example, shoot growth and seed yield exposed to drought stress were increased by 8% when 3 kg ha^{-1} GB was applied to soybean (*Glycine max* L. Merr.) (Agboma *et al.*, 1997b). There has also been indications of GB treatment induced increases in mean above-ground biomass production and grain yield up to 25% in maize (*Zea mays* L.), and by up to 11% in sorghum [*Sorghum bicolor* (L.) Moench] when plants were suffering from drought. Increase in grain yield was due to increased grain set and reduced abortion, i.e. more grains per head whereas single grain weight was unaffected (Agboma *et al.*, 1997a). Furthermore, field experiments conducted in California have indicated that the fruit yield of tomato plants increased by 29-39% under heat and salt stress when GB was applied during the mid flowering stage. Similar trends with approximate increase of 20-30% have been obtained in experiments conducted in the greenhouse of commercial vegetable grower in Finland but with unstressed tomato plants (Mäkelä *et al.*, 1998a).

The explanation to the yield increases of stressed tomato plants after application of GB has been proposed to lie at least partly in the increased net photosynthesis and decreased rate of photorespiration. Moreover, increased stomatal conductance (Mäkelä *et al.*, 1998b) might have induced more efficient gaş exchange and thus, better availability of carbon for photosynthetic processes and ability to avoid possibly photoinhibition (Mäkelä *et al.*, 1999)without any disturbance in ABA content (Mäkelä *et al.*, 1998b). According to Bergmann and Eckert (1984) 20 mM application of glycinebetaine to roots increased the net photosynthesis and water use efficiency of winter wheat, resulting in increased number of fertile florets and grains per plant. Similar results on increased net photosynthesis have been obtained with stressed tomato (*Lycopersicon esculentum* Mill.) and turnip rape (Mäkelä *et al.*, 1999). Actually, the sustainability of photosynthetic efficiency achieved with exogenous GB has been explained later by GB induced maintenance of chloroplast ultrastructure (Mäkelä *et al.*, 2000) and chloroplast volume (Rajasekaran *et al.*, 1997). Furthermore, Whapman *et al.* (1993) and Blunden *et al.* (1997) reported increased chlorophyll content in plants treated with seaweed extract containing GB. However, the mechanism to these changes is not known even yet. Several studies with bacteria and isolated chloroplasts indicate that GB protects, stabilizes and activates the proteins of photosynthetic reactions (Papageourgiou and Murata, 1995).

GLYCINEBETAINE FOR INDUSTRIAL PURPOSES

Due to the unique nature of GB molecule it has been exploited in several industrial applications developed in recent years, such as in pharmaceuticals, cosmetics, fermentation and cell cultures (Fig. 3). In the cosmetic industry there is an increasing demand for safety, non-irritating and non-sensitising raw materials. GB is non-mutagenic and non-allergenic compound which has an amphoteric character and conditioning properties. Moreover, it has a moisturizing effect by improving moisture retention of the skin and produces reduction in the irritation potential. Since, the molecule is non-toxic, hygroscopic and osmoprotective its properties are desired. Accordingly, it is nowadays used in skin creams and ointments, medicated cleansers, syndets, after shave lotions and deodorants.

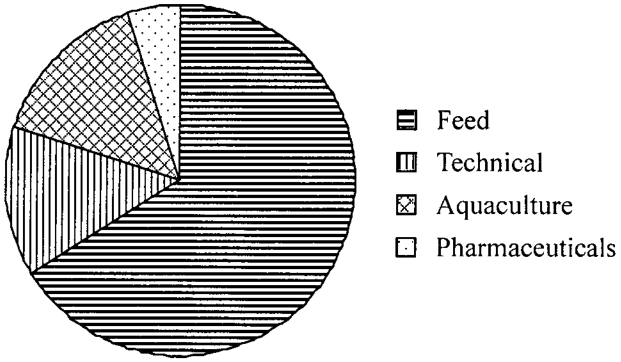

Fig. 3 : The utilization of GB (produced by Danisco in Finland) for different purposes in agriculture and industry.

Traditionally GB has been used in pharmaceutical industry primarily to prevent disturbances in liver methionine metabolism. This is typically a result of e.g. excess intake of alcohol causing fat accumulation into liver, i.e. so called 'fatty liver' (Barak *et al.*, 1993). Another application of GB in pharmaceutical industry is in a treatment of a metabolic disease 'homocystinurea' caused by an inefficient recirculation of homocysteine to methionine. Besides the disease many people have elevated levels of homocysteine in the blood which may increase the risk for coronary diseases (Dudman *et al.*, 1993). Most mammalian membranes contain choline which is probably an essential nutrient for humans and acetylcholine present in neurons and placenta as a neurotransmitter. GB is a choline metabolite and important methyl donor for the regeneration of methionine from homocyateine. A deficiency of choline in neonates leads to a respiratory distress syndrome in premature infants. Infants usually get choline in the milk of maternal blood. Thus, GB could be added to the milk in order to avoid choline deficiency. Moreover, supplemental choline exposure during the prenatal period has been reported to alter permanently the brain structure and improve the memory function of rats (Rohlfs *et al.*, 1993).

In the fermentation industry, GB is used as a methyl donor and metabolic regulator. It has been shown to relieve osmotic stress and improve yield of lysine-producing *Brevibacterium lactofermentum* (Kawahara *et al.*, 1990). It has been possible to increase the survival and growth rate of bacteria adding GB to the culture medium (Kets and de Bont, 1994). The role of GB has been putative also in embryo cultures, as these are needed in breeding programs. As GB addition has increased the growth and survival rate of cultured and isolated cells, GB is used widely as a practice in cell isolation and culture media nowadays.

CONCLUSIONS

GB, an additional product from sugar beet processing industry, has for long been used in applications of pharmaceutical and feed industry. The use of GB in animal feed is based on its metabolic role in animals both as methyl donor and an osmolyte. Supplemental GB can help animal cells to maintain osmotic balance when exposed to heat and other osmotic disturbances. Another application of GB studied intensively during the last decade is the possibility to enhance crop growth and productivity under water, salt and cold stresses. The physiological basis of yield increases is still under investigation but there are implications that GB might affect the reproductive sterility of cultivated plants. The data indicates also that plant species and even cultivars differ in response to foliar-applied GB. Environmental conditions and plant species also affect the uptake and translocation markedly but environmental factors interact also with genotype causing differences in crop's responsiveness to applied GB. In conclusion, the previously called waste product and processing problem, GB, has proved to be very worthy in comparison to the sugar itself.

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