Photocontrol of Boron Metabolism in Sea Grasses

Abstract. *Boron is taken up in the dark and is excreted in response to light by the leaves of the marine vascular plant,* Halophila engelmannii *Ascherson. This phenomenon makes possible the study of boron metabolism in a higher plant without the stress of boron deficiency. Dependence of boron excretion on photosynthetic CO_2 fixation suggests that an organoboron complex between boron and a newly formed carbon compound from photosynthesis is selectively excreted.*

The function or functions of boron in higher plants has been debated since Warington discovered that it was essential for growth of broad beans. Its proposed physiological functions include regulation of phenolic acid biosynthesis, carbohydrate metabolism, nucleic acid metabolism, cell wall metabolism, cell division, responses to plant growth regulators, and translocation of organic compounds (*1*). Changes in these diverse areas of metabolism have all been shown to be produced during boron deficiency (*2*). However, since some of these symptoms also occur in plants with deficiencies of other mineral elements, it has been difficult to pinpoint the primary site of boron involvement in cell metabolism (*2*).

In this report I describe a higher plant system in which a readily detectable shift in boron metabolism occurs without subjecting plants to the abnormal physiological stress of boron deficiency. I present evidence for the photocontrol of leaf tissue boron in the marine flowering plants known as sea grasses (*3*). The regulation of tissue boron by light, heretofore unreported, makes boron metabolism amenable to experimental study under normal growth conditions. The observations presented are consistent with the hypothesis that boron participates in sea grass metabolism as a complexing agent for organic material which is selectively excreted from leaves in the light.

Cultures of a tropical sea grass, *Halophila engelmannii* Ascherson, were grown under laboratory conditions, rooted in natural bay sediments, with seawater at a salinity of 30 parts per thousand and a photoperiod of 12 hours of light and 12 hours of dark. Light and temperature were held constant (see legend in Fig. 1A). Plant leaves were covered by water to a depth of 6 to 10 cm. A stock culture has been propagated in this way for more than 2 years.

Approximately a 50 percent increase in boron content (*4*) of *Halophila* leaves was observed upon switching plants from light to darkness (Fig. 1A). The opposite pattern was observed when *Halophila* was transferred from dark to light conditions. The kinetics of the process indicate a steady increase or decrease in leaf boron accomplished within 2 to 3 hours, although the light-induced decrease seemed to lag about 30 minutes, in contrast to the dark-induced increase.

Other ion concentrations changed at the same time that boron was changing. Potassium and chloride were accumulated in response to light (Fig. 1B), as was observed for the green alga *Nitella* (*5*) and the freshwater plants *Elodea* and *Vallisneria* (*6*). Leaf sodium remained constant. However, boron fluctuation in *Halophila* did not appear to be simply ion exchange linked to light-induced K^+ and Cl^- flux. The increase in leaf tissue Cl^- was approximately 1000 μeq/g, while the decrease in boron was only about 100 μeq/g.

A similar pattern of boron fluctuations was observed in a natural population of the sea grass *Halodule wrightii* Ascherson, growing in Redfish Bay, Texas. Diurnal sampling of leaves was carried out starting before dawn, and the boron content was assayed. Boron content decreased from > 800 μg /g (dry weight) in the dark (before dawn) to < 600 μg /g by midday, and again returned to the high level by 3 hours after sunset. No correlation existed between boron content and salinity of the surrounding bay water.

An experiment was performed to distinguish between loss of boron from the leaves to the water or transport to other parts of the plant. *Halophila* shoots were cut off from the rhizome, maintained in darkness for 12 hours, and then exposed to light. The boron content of excised shoots so treated still decreased over a 5-hour period (Fig. 2, control curve, no

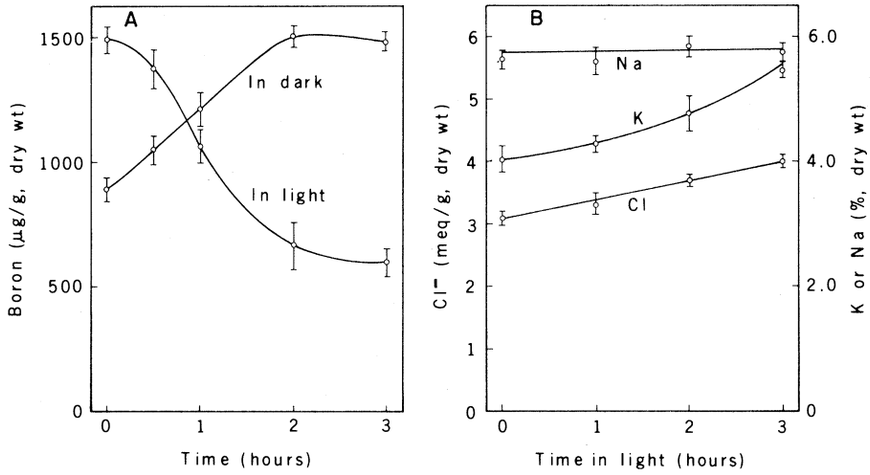

Fig. 1.(A) Change in boron content of *Halophila engelmannii* leaves in darkness and in light. The bars indicate the standard deviation for four replicates. Before the lights were turned out, intact plants were illuminated by either Cool-White or Agro-lite fluorescent lamps (Westinghouse) at 40 to 50 μE/m^2 per second for 12 hours and held at 26° to 28°C. Prior to illumination with the same lights, intact plants were held in the dark for 12 hours. Response was the same with both types of fluorescent lights. Plants were submerged in 8 to 10 liters of seawater and periodically agitated during the course of experiments. At the times indicated, shoots were cut off from the plant and assayed for boron. (B) Change in Cl^-, K^+, and Na^+ content of *Halophila engelmannii* leaves upon illumination as in (A). The bars indicate standard deviation for four replicates. Results were obtained from a dark to light experiment similar to that described in (A). The K^+ and Na^+ were determined by atomic absorption spectroscopy; Cl^- was measured by titration with mercuric nitrate.

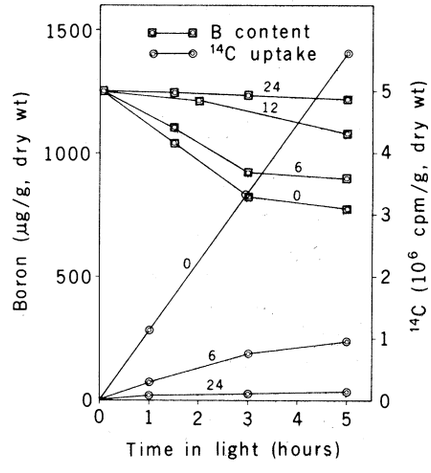

Fig. 2. Effect of DCMU on $H^{14}CO_3^-$ assimilation and boron efflux by *Halophila engelmannii* leaves. Each point represents the average of two duplicate samples. DCMU was present, dissolved in filtered off-shore seawater, at concentrations of 6, 12, and 24 μM. Control curves are marked 0. Shoots were excised and kept in darkness 12 hours prior to illumination. Incorporation of ^{14}C for control leaves is equivalent to a photosynthetic rate of 1.7 μg of carbon per milligram of tissue (dry) per hour.

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DCMU). Thus, the decrease in boron in the leaf tissue of *Halophila* is considered the result of boron efflux from the leaves to the water.

Bowen (*7*) has shown that inorganic boron, probably borate, is accumulated by excised sugar cane leaves and forms complexes with sugars and other metabolites in the cytoplasm. In view of this result, it seems likely that borate is absorbed by the sea grasses from seawater in darkness. However, boron efflux could represent either excretion of borate, after dissociation from organic compounds within the plant, or excretion of organically complexed boron. The kinetics of the efflux process (Fig. 1A; rapid decrease after a 30-minute lag) suggest that a specific organoboron complex may be produced after illumination begins and then it decreases within the cells under continued illumination. The identification of such an organic material, possibly by chromatographic techniques, would help answer the question of the chemical form of boron in plants, a central issue in the overall problem of boron function in plants. Whether this form is, as others have suggested (*1*, *2*), a polyhydroxy compound with *cis*-hydroxyl groups (for example, a carbohydrate or polyphenol) or some other chemical species is not known.

In order to examine the relation between boron efflux and photosynthetic activity, excised shoots were treated with the inhibitor, DCMU [3-(3,4-dichlorophenyl)-1,1-dimethylurea], which prevents CO_2 fixation by interfering with photosystem 2 activity (O_2 evolution) (*8*). Metabolic energy (adenosine triphosphate) can still be produced by the processes of cyclic photophosphorylation or respiration. When dissolved in seawater at an external concentration of 6 μM, DCMU inhibited boron efflux, compared to the control, by 24 percent, while photosynthetic carbon fixation was reduced by 77 percent (Fig. 2). Progressively more inhibition of boron efflux, concomitant with inhibition of $^{14}CO_2$ fixation, was observed with increasing DCMU concentrations, such that 24 μM DCMU produced essentially complete inhibition of both processes within an hour. These results would occur if some newly formed organic compound (photosynthate) were the source of organic carbon which formed complexes with boron.

Sea grasses, as demonstrated by *Halophila* and *Halodule*, would seem to offer advantages to elucidating the primary function of boron in higher plants. Since leaves of most other higher plants are not bathed in water, light-induced fluxes of boron, necessarily limited to within these plants, would be much more difficult to observe. However, in such plants, compartmentalization of boron in specific leaf tissues, or translocation to and from various plant parts, may be mechanisms under photocontrol, which regulate boron metabolism.

This information may also have a bearing on the restriction of sea grasses to growth in salt water. Boron, at 4.5 μg/ml, is about 500 times more abundant in salt water than in freshwater; and it could represent an exaggerated nutritional requirement for these salt-tolerant angiosperms.

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References and Notes

1. E. H. Birnbaum, W. M. Dugger, B. C. Beasley, *Plant Physiol.* **59**, 1034 (1977); W. M. Dugger, in *Trace Elements in the Environment*, E. L. Kothny, Ed. (American Chemical Society, Washington, D.C., 1973), p. 112–129.
2. E. J. Hewitt and T. A. Smith, *Plant Mineral Nutrition* (English Universities Press, London, 1975), pp. 248–251; E. Epstein, *Mineral Nutrition of Plants: Principles and Perspectives* (Wiley, New York, 1972), pp. 303–305.
3. A comparative survey of boron contents of aquatic plants revealed that five species of southern Texas sea grasses (families Hydrocharitaceae and Potamogetonaceae) contained much higher levels of boron (dry weight) than seven marine macroalgae (*Enteromorpha, Ulva, Padina, Dictyota, Gracilaria, Centroceras*, and *Rhodymenia*), three salt-marsh halophytes (*Spartina alterniflora, Sporobolus virginicus*, and *Salicornia bigeloveii*), and four freshwater macrophytes (*Elodea, Vallisneria, Ceratophyllum*, and *Potamogeton*). The boron content of sea grasses ranged from 350 to 1000 μg/g (average, 625), whereas the organisms with the next highest concentration of boron were marine algae, showing 50 to 270 μg/g (average, 175). Boron in the three halophytes averaged 36 μg/g, and the freshwater plants averaged 40 μg/g. On the basis that the dry weight was equal to 15 to 20 percent of the fresh weight, the boron content of sea grasses represents a 20- to 25-fold concentration of boron over the 4.5 mg/liter usually found in seawater.
4. For analysis, single shoots, each consisting of a portion of the stem and six to seven leaves, were harvested at random from one rhizome and dried at 90°C. Boron was assayed by a colorimetric method [W. T. Dible, E. Truog, K. C. Berger, *Anal. Chem.* **26**, 418 (1954)] with the use of the curcumin reagent. Plant material was dry-ashed in the presence of $Ca(OH)_2$ at 550°C. Interference by NO_3^- and Fe was checked and was insignificant.
5. D. R. Hoagland and A. R. Davis, *J. Gen. Physiol.* **6**, 47 (1923); E. A. C. MacRobbie, *Aust. J. Biol. Sci.* **19**, 363 (1966).
6. R. N. van Lookeren Campagne, *Acta Bot. Neerl.* **6**, 543 (1957); W. D. Jeschke, *Planta* **91**, 111 (1970).
7. J. E. Bowen, *Plant Cell Physiol.* **9**, 467 (1968).
8. G. Gingras, C. Lemasson, D. C. Fork, *Biochim. Biophys. Acta* **69**, 438 (1963); S. Izawa and N. Good, *Methods Enzymol.* **24**, 359 (1971).
9. I thank J. Suter and C. Webre for technical assistance and T. Moore for drafting the figures. This is University of Texas Marine Science Institute contribution 246.

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Rejection of Male Skin Grafts by Splenectomized Female Mice

Abstract. *Female mice of the C3H strain normally do not reject skin grafts from males of the same strain; however, 40 percent of splenectomized C3H female mice completely rejected C3H male skin grafts applied 2 weeks later. All splenectomized females showed at least transitory signs of graft rejection.*

In some inbred strains of mice, females reject skin grafts from males of the same strain (*1*). In other strains, including C3H, male to female grafts survive indefinitely (*2*). Nevertheless, C3H/An females that have accepted C3H male skin grafts produce antibody to the H-Y antigen (anti-H-Y), that is demonstrable by reaction with male but not female cells. Furthermore, some C3H/An females that have accepted one male skin graft may reject a second applied 3 to 6 weeks after the first (*3*). Thus, the survival of C3H/An male skin on C3H/An females is not due to failure of the grafts to elicit a response to H-Y. It has been suggested that anti-H-Y might actually protect the grafts (*3*), as in immunological enhancement of tumor allografts (*4*, *5*).

Table 1. Rejection of C3H male skin grafts by splenectomized C3H females.

Treatment of C3H female recipients	Mice grafted (No.)	Crisis*	Rejected†	Day of rejection
Splenectomy‡	15	9	6	45,61, 70,91, 91,100
Sham-splenectomy	10	0	0	
None	30	0	0	

*Grafts underwent transitory signs of apparent rejection during which scabs appeared. These grafts ultimately returned to a healthy state with a full crop of tail hair, although they were diminished in size. †Day when no sign of the graft remained. ‡Female recipients were either splenectomized or sham-splenectomized 2 weeks prior to receiving a male skin graft.

Prehn (*6*) has reported that the successful transplantation of tumor allografts was reduced when the recipients had been splenectomized. This might be construed as immunological enhancement, and suggested that splenectomy might facilitate the rejection of male skin grafts by C3H/An females. Although there were no outright rejections, C3H male skin grafts underwent a transitory crisis in splenectomized females that was

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