Study on The Effects of Boron on The Structure of Chloroplasts of Cauliflower , By Using Light Scattering Technique

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Abstract

Effects of Boron on the structure of chloroplasts membrane isolated from cauliflower are investigated , using light scattering technique. Results obtained in this study suggest that Boron in the concentration range (0.1-5 µm) can fluidize the lipids of the chloroplast membrane due to different extent. Mechanisms by which Boron can change the lipid fluidity is discussed. Furthermore, an experimental evidence is presented to show that2µM Boron can mediate conformational changes in the membrane –bound proteins of the cauliflower's chloroplast.

Introduction

Although the essentiality of Boron for higher plants is well established , the mechanisms of its action are far from being well understood. It has been indicated that Boron is involved in carbohydrate metabolism, translocation, protein synthesis , phenolic production , and pollen germination.

In an attempt to determine the primary role of Boron in plants, Parr and loughman (1) reported that one aspect which could underlie all types of responses to Boron is that of impaired membrane function. One of the well known Boron deficiency symptoms is the reduction in capacity for Ion transport. Robertson and Loughmann (2) showed that the reduced capacity for phosphate absorption could be restored almost completely by supplying 10µM H_3BO_3 for 0ne hour. Tanada(3) found out that induced a bioelectric field change the hypocotyl tissues in mung beans. He attribute these changes to modification of some membrane components. Even though the control of membrane function by Boron is beyond doubt, its exact involvement in membrane component is not yet understood .Thus in this study an attempt has been made to determine at a molecular level the direct effect of Boron upon the components of chloroplast membrane as well as the concentration at which Boron maintaining the structural integrity of the membrane.

Material and Methods

Isolation of chloroplast: 10 g of mature clean cauliflower leaves cut into small pieces ground in a pistil and mortar(kept in an ice bucket) using 50 ml of cold tris buffer (5x10 M tris pH 7.8) . The homogenate were filtered through three layers of gauze cheese cloth. The filtrate were centrifuged at 200xg for 10 min. the supernatant were again centrifuged at 1000xg for 15 min. The pellet were collected and purified by resuspended with 5 ml of tris

buffer, then 1 ml of this suspension were added to sucrose gradient tube(1.6,1.0,0.5,0.25M), and stored for 2 hours . Pure chloroplasts were collected somewhere nearby region of 1.3 M concentration.

Sample preparation: The stock suspension of the isolated chloroplast was diluted by aqueous solution 3 ml samples of the diluted chloroplast suspension were then prepared. Various concentrations of aqueous solutions of Boric acid were prepared.

To prepare the sample for turbidity measurement, 1ml of certain solution was added to the chloroplast suspension; the sample was allowed for 3minutes at room temperature, turbidity was measured by Lovibond turbidometer.

Light scattering measurement: Light scattering intensity at 90 (190) was measured by light scattering set up. The set up consists of He-Ne laser as light source , the sample holder calibrated with different angles , photomultiplier, digital voltmeter, and water bath for temperature measurement.

Results and Discussion

It becomes fact that function of any biological system can be greatly affected by variations in the structure of that system. Likewise, many functional aspects of cell membrane have been shown to be impaired or modified by alteration in the structure of the membrane.

Lipid fluidity plays a very important role in maintaining the proper functions of the cell membrane. Many membrane – bound enzymes require certain degree of lipid fluidity for their optimum activities. In addition, the rate of transport throughout the membrane is highly dependent upon the amount of lipid fluidity. Convincingly, Boron seems to be essential for proper functions of cell membrane in higher plants. The activities of many membrane – bound enzymes have been shown to be markedly affected by Boron deficiency (6,7). Transport of potassium, phosphorus, and Rb appears to be significantly altered by the action of Boron (1). Also, Boron has shown to facilitate the permeability of water throughout the liposomes (1).

Yes, it is not clear whether the Boron – induced functional changes in the plant cell membranes could be attributed to a direct interaction of Boron with the membrane components or might be mediated by some other actions of Boron.

Finding presented in this study provide experimental evidence required for the important conclusion that Boron may interact directly with chloroplast membrane components. A decline in the turbidity of the chloroplast suspension , shown in figl , indicates that Boron in the concentration (0.1-4μM) could be fluidize the membrane lipids. Boron in the concentration range (o.1-4μM) seems to be incorporated in the chloroplast membrane , perturbing the lipid packing and increasing the lipid fluidity. This comes in agreement with a previous work done on different membrane(8). Therefore, Boron might be considered as regulator of membrane fluidity acting like cholesterol in the animal cell membrane (9).

Furthermore , results illustrated in fig 3 clearly indicate that 2μM of Boron may act to induce thermal destabilization of membrane –bound proteins of the chloroplast as result of structural changes. The observed structural changes in the membrane –bound proteins of chloroplast that caused by 2μM boron might com as a result of a direct interaction of Boron with the membrane –bound protein or might be due to the Boron – induced lipid fluidity changes.

However, results shown in fig 3 rules out the first possibility that Boron could interact directly with membrane –bound proteins since boron seems to fluidize the lecithin liposomes in spit of the absence of the membrane – bound proteins.

Interestingly, Boron with high concentration (4- 10µM) seems not to be incorporated in the chloroplast membrane, since there are no effects could be observed on chloroplast membrane. Therefore, it is likely that Boron with high concentration could translocate through the chloroplast membrane , mediating the toxicity of the cell. Toxicity of high level of Boron applications to plants has been well known.

In order to analyze the temperature –dependence of 190 accurately , differentiation of 190 with respect to temperature (d190/dt) was accomplished by computer program. The temperature – dependent plots of (d190/dt) with 2µm Boron and without Boron are depicted in fig 4. The profile without Boron consists of two bands that start at 50˚C and 73˚C characteristic temperatures.The presence of 2µM Boron appears to shift this profile toward the lower temperature range by about 5˚C.

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تأثير عنصر البورون في تركيب غشاء البلاسيدات الخضر لنبات القرنابيط باستعمال تقنية تشتت الضوء

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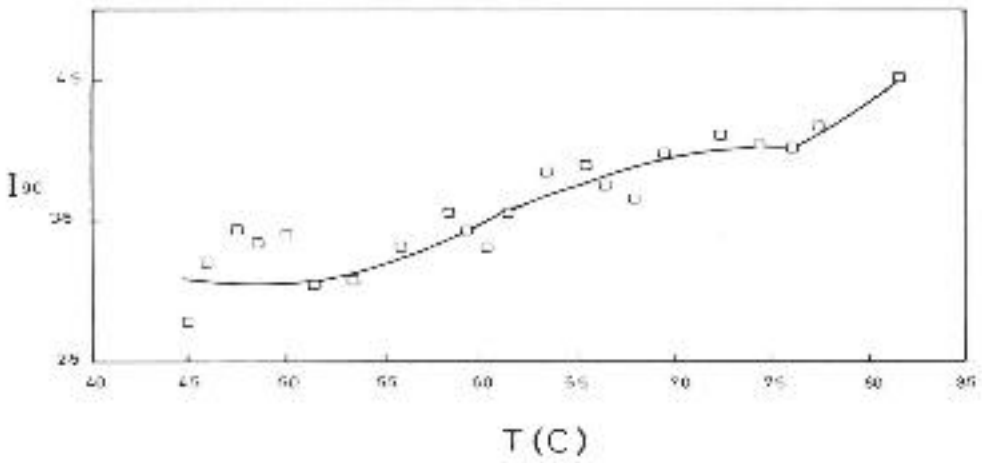
الخلاصة

تضمن البحث دراسة تأثير عنصر البورون في بناء وعمل غشاء البلاسيدات الخضراء المعزولة من نبات القرنابيط باستعمال تقنية تشتت الضوء. اظهرت النتائج ان البورون بتراكيز (0.1 – 5 مايكرومول) ادى الى سيولة دهون الغشاء بدرجات متفاوتة فضلا عن ذلك تم التوصل الى ادلة تجريبية اوضحت ان تركيز البورون (2 مايكومول) ادى الى تغيرات في هيئة بروتين الغشاء حفاظا على نفاذية اغشية البلاسيدات .



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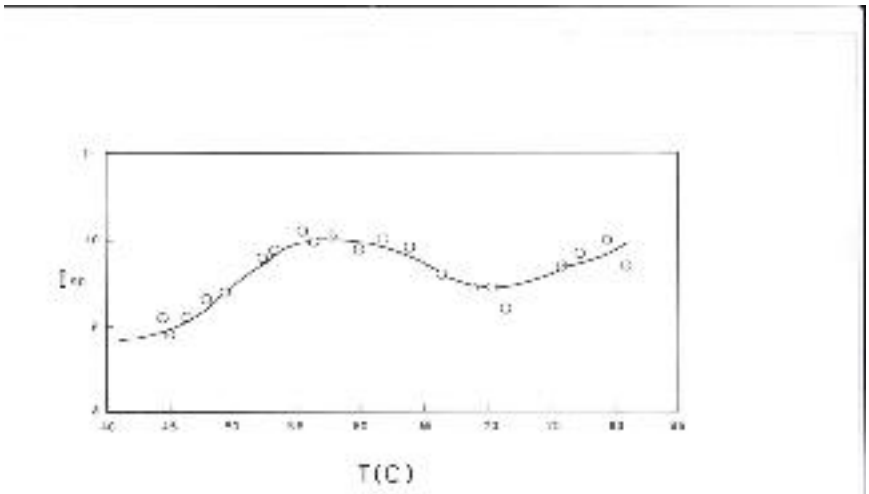

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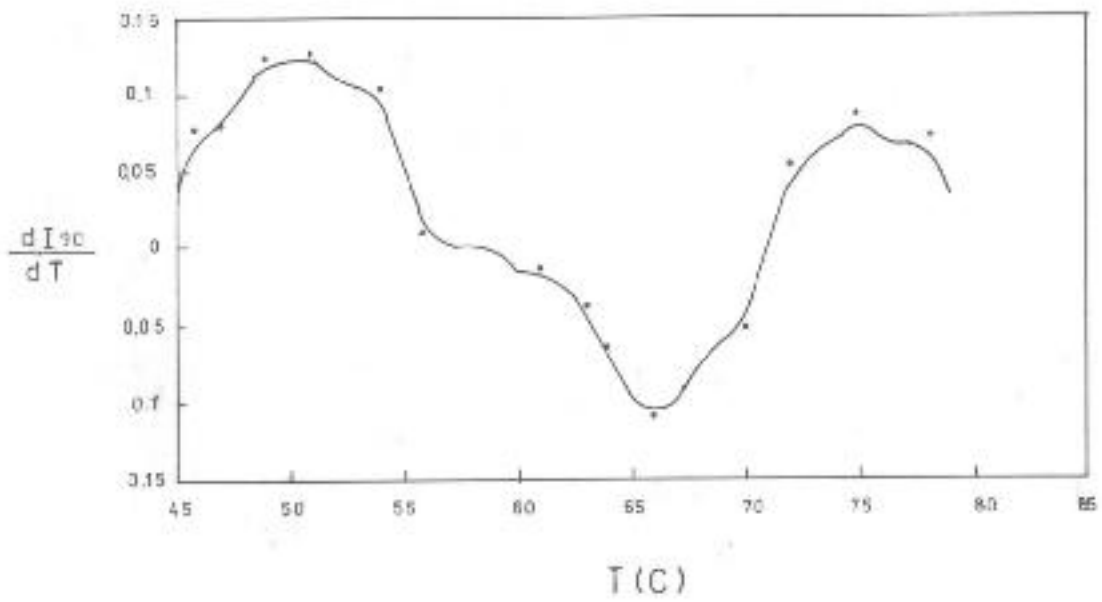

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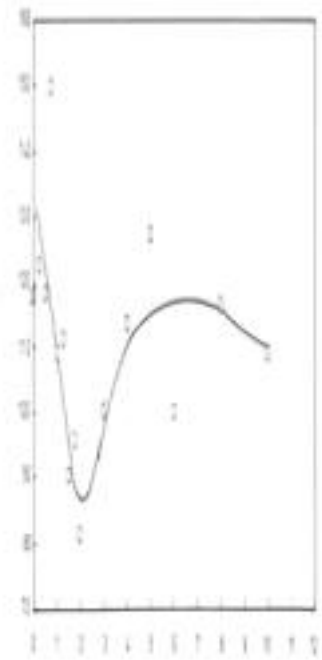

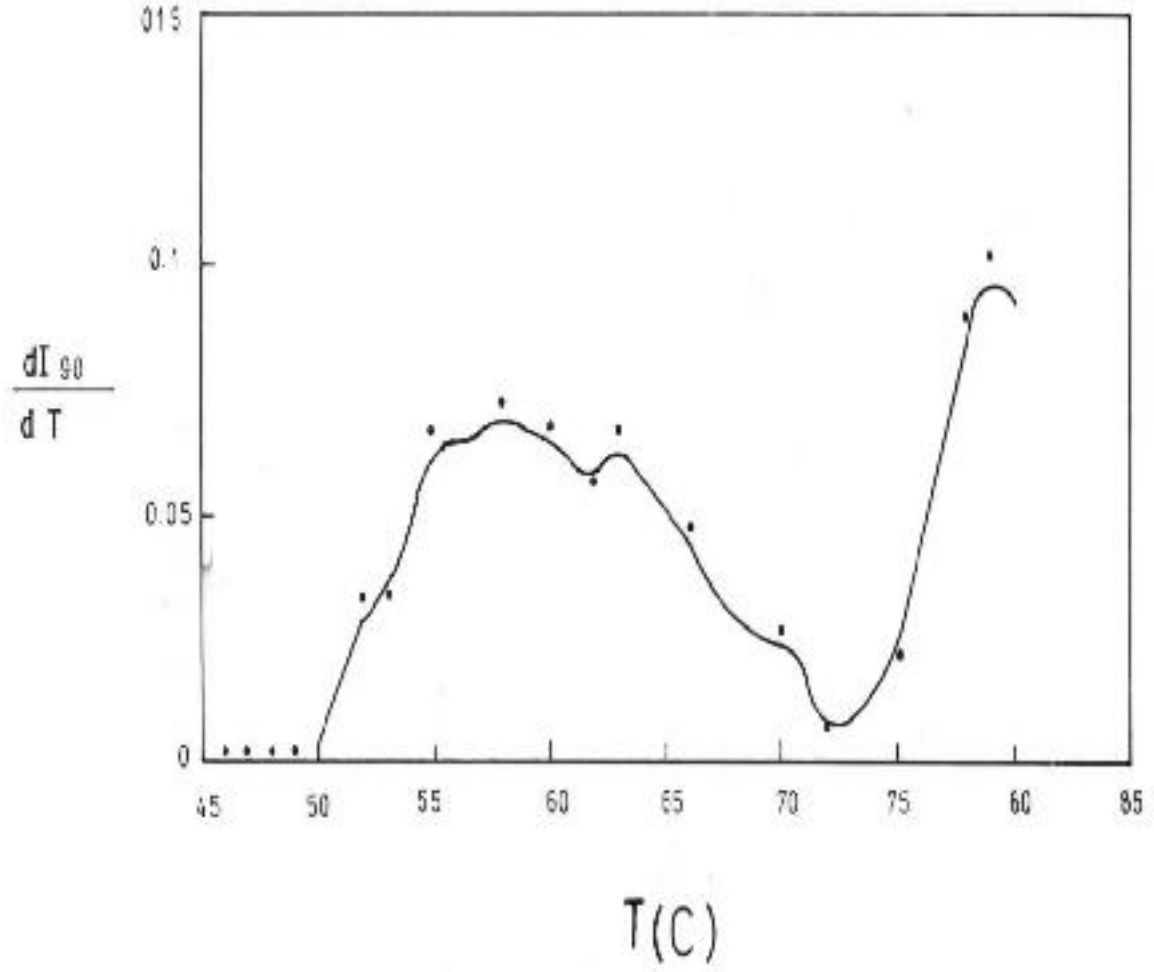

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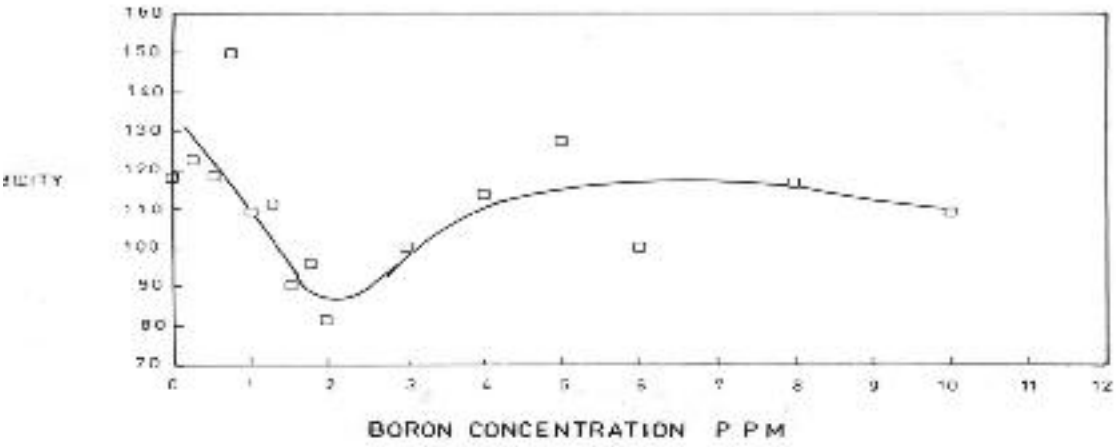

Fig(1):Turbidity of cauliflower chloroplast with different Boron concentration

Fig2:Temperature –dependent plot of light scattering intensity at angle of 90 degree (190) for cauliflower chloroplast suspension

(a)Without Boron
(b)With 2µM Boron

Fig(3): Turbidity of lecithin liposomes containing different Boron concentration

Fig 4: Temperature –dependent plot of (d 190/d T) for cauliflower chloroplast suspension

(a) Without Boron

(b) With 2µM Boron.