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Permeability of Boric Acid Across Lipid Bilayers and Factors Affecting It

C. Dordas,* P.H. Brown
Department of Pomology, University of California, One Shields Avenue, Davis, CA 95616, USA

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Abstract. Boron enters plant roots as undissociated boric acid (H_3BO_3). Significant differences in B uptake are frequently observed even when plants are grown under identical conditions. It has been theorized that these differences reflect species differences in permeability coefficient of H_3BO_3 across plasma membrane. The permeability coefficient of boric acid however, has not been experimentally determined across any artificial or plant membrane. In the experiments described here the permeability coefficient of boric acid in liposomes made of phosphatidylcholine was 4.9×10^{-6} cm sec^{-1}, which is in good agreement with the theoretical value. The permeability coefficient varied from 7×10^{-6} to 9.5×10^{-9} cm sec^{-1} with changes in sterols (cholesterol), the type of phospholipid head group, the length of the fatty acyl chain, and the pH of the medium. In this study we also used *Arabidopsis thaliana* mutants which differ in lipid composition to study the effect of lipid composition on B uptake. The *chs1-1* mutant which has lower proportion of sterols shows 30% higher B uptake compared with the wild type, while the *act1-1* mutant which has an increased percentage of longer fatty acids, exhibited 35% lower uptake than the wild type. Lipid composition changes in each of the remaining mutants influenced B uptake to various extents. These data suggest that lipid composition of the plasma membrane can affect total B uptake.

Key words: Boric acid — Permeability — Uptake — Boron — Liposomes — *Arabidopsis thaliana*

* *Present address:* Plant Science Department, Faculty of Agriculture and Food Science, University of Manitoba, Winnipeg MB R 3T 2N2, Canada

Correspondence to: P.H. Brown

Introduction

Boron is an essential element for vascular plants, and also for diatoms, cyanobacteria and a number of species of marine algal flagellates (Loomis & Durst, 1992; Marschner, 1995 and reference therein). There is no conclusive evidence that B is essential in bacteria (except from Cyanobacteria), fungi and green algae. Recently there has been increasing evidence for the essentiality of B in animals, especially zebrafish (*Danio rerio*), trout (*Oncorhynchus mykiss*) (Rowe et al., 1998) and frogs (*Xenopus laevis*) (Fort et al., 1998).

Boron is absorbed from the soil solution by roots mainly as undissociated boric acid (H_3BO_3) which is a very weak Lewis acid ($H_3BO_3 \rightarrow B(OH)_4^- + H^+$ pKa = 9.25, 25°C Greenwood, 1973). At physiological pH values H_3BO_3 is a small noncharged molecule with a molecular radius of 2.573 Å, which is similar to urea (2.618 Å) and other small nonelectrolytes. Boric acid (as H_3BO_3) is very soluble in water, with a saturation solution concentration at 20°C of 0.75 M, and an ether/water partition coefficient of 0.035 (Raven, 1980). The calculated permeability coefficient of plant membranes to H_3BO_3 based on the partition coefficient (0.035) is 8×10^{-6} cm sec^{-1} (Raven, 1980). Based upon this calculation Raven proposed that B uptake must be a passive process. This calculated coefficient has not, however, been verified in any biological systems or artificial lipid bilayers. The permeability coefficient of nonelectrolytes through membranes also varies with lipid composition (concentration of sterols, head group, and fatty acyl chain length), physical properties of the membrane, and pH, especially for weak acids (Wolosin & Ginsburg, 1975; Wolosin et al., 1978; Xiang & Anderson, 1994; Lande et al., 1995; Paula et al., 1996).

The mechanisms of B uptake remain a controversial subject, and there is evidence supporting both active and passive uptake. Despite the controversy, passive uptake is currently the most widely accepted mechanism of B uptake in plants (Nable, 1988; Nable & Paull, 1991; Brown & Hu, 1994; Hu & Brown, 1997; Nabel et al., 1997).

The evidence for passive B absorption at normal and high B concentrations is quite unambiguous. Nevertheless there is still considerable species variation in B uptake that is difficult to reconcile. Nable (1988) found that the B concentration and total B content in all organs of five barley and six wheat cultivars differed dramatically even though all were grown under identical conditions. For example, barley cultivars 'Sahara 3763' and 'Schooner' accumulated 112 and 710 mg B kg^{-1} dry weight in the youngest expanded leaf blade, respectively, which is more than a 6-fold difference in B accumulation. These differences in B uptake cannot be explained by differences in water use, as reported water use efficiency in 13 wheat cultivars only varies from 3.1 to 4 g dry matter Kg $water^{-1}$ (Passioura, 1977). The major mechanism of tolerance to high B in crop plants (e.g., wheat, barley) is the restriction in B uptake (Nable, 1988; Nable & Paull, 1991; Nable et al., 1997). It has been speculated that the difference in B uptake between plant species and cultivars is likely due to variations in membrane permeability, although this has never been verified in any plant species (Huang & Graham, 1990; Nable & Paull, 1991; Hu & Brown, 1997).

The permeability coefficient (P) of H^+, K^+, H_2O differed by up to two orders of magnitude with changes in length of the fatty acid chain (Paula et al., 1996). While for P_{urea} and $P_{glycerol}$ the difference was about 6-fold. Striking differences in permeability of urea were also found between different types of lipids and with changes in lipid composition. Urea permeability was three orders of magnitude slower in 60% sphingomyelin and 40% cholesterol as compared with dilinoeyol lecithin (Lande et al., 1995). Similar data were reported for acetamide and for NH_3 which varied by two orders of magnitude, and about 15-fold, respectively (Lande et al., 1995). In *Escherichia coli* it was found that lipid composition affects the glycerol uptake (Truniger & Boos, 1993). In this study they observed that mutants that were defective in PE synthesis had 3-fold lower glycerol uptake than the wild type.

The objective of this study was to measure the permeability coefficient of H_3BO_3 across lipid bilayers and to compare this permeability with the permeability of other similar compounds such as urea, glycerol and water. This study also aims to determine the effect of pH, lipid composition and on the permeability of boric acid across artificial lipid bilayers. Furthermore, we tested the hypothesis that different mutants of *Arabidopsis thaliana* differing in lipid composition would differ in B uptake.

Materials and Methods

Chemicals

All lipids were purchased from Avanti Biochemicals (Birmingham, AL). All other chemicals were bought from Sigma Chemicals (St. Louis, MO). The water was purified using a Barnstead NANOpure system (Dubuque, Iowa).

Measurement of the Permeability Coefficient of Boric Acid and Other Nonelectrolytes

Vesicle Preparation

Liposomes were prepared as follows: 1 ml of egg phosphatidycholine (20 mg/ml) was placed in a tube and was dried under N_2, then kept under vacuum for about 2 hr. One ml of buffer solution containing 10 mM PIPES (pH = 7) and 50 mM K_2SO_4 was then added. The tube was vortexed in an atmosphere of nitrogen, subjected to ten cycles of freezing and thawing in liquid nitrogen and warm water, and then extruded 21 times using a Liposofast extruder (Avestin, Ottawa, Canada) with two polycarbonate filters (Osmotics, CA) of a pore diameter of 200 nm to obtain unilamellar vesicles. The size of the vesicles was determined with dynamic light scattering, using a BI-90 particle sizer (Brookhaven Instruments, Holtsville, NY).

Stopped Flow Measurements

The stopped flow measurements were made in an apparatus that was manufactured according to Colowick and Kaplan (1960) and tested using the method of Tonomara et al. (1978). The dead time was 24 msec. Osmotic water permeability was measured by recording the time course of the change of light scattering at 465 nm wavelength. The size of the vesicles was determined before and after mixing to ensure that rapid mixing did not lead to vesicle rupture or fusion. The permeability coefficients of boric acid and other nonelectrolytes such as urea and glycerol and osmotic water permeability (P_f) for water were determined as the change of liposome volume caused by imposition of a transmembrane osmotic gradient using methods described previously (Verkman et al., 1985; Van Heeswijk & van Os, 1986; Ye & Verkman, 1989; De Gier, 1993).

Light scattering at 465 nm was recorded in an OLIS-RSM rapid kinetics spectrophotometer (OLIS, GA) at 90° angle. For water measurements the data acquisition system was set at one determination per msec and for the nonelectrolytes it was set at one determination each 16 msec. All measurements were performed at room temperature. Equal volumes of liposome suspension prepared as described previously with a concentration of 1 mg/ml phospholipid were mixed with buffer containing 400 mM H_3BO_3, 200 mM sucrose, 400 mM glycerol or 400 mM urea. For the determination of osmotic water permeability (P_f) these liposomes were exposed to 200 mM sucrose to induce an outward osmotic gradient. The observed time course change in light scattering corresponds to the time course change in volume of the vesicles. The scattering data were fitted to a single exponential function (Verkman et al., 1985; Van Heeswijk and van Os, 1986). The permeability coefficient was calculated as follows:

$$P_f = k\, V_o/(V_w\, A\, \Delta c) = kr/(3V_w\, \Delta c) \quad (1)$$

Where k is the the time constant of the exponential curve, V_w is the molar volume of water (18 cm^3/mole), V_o is the internal volume of the vesicles, A is the surface area of the vesicles, r is the radius of the vesicles, and Δc is the osmotic gradient.

The permeability coefficient for H_3BO_3, urea, and glycerol was determined according to Verkman et al., (1985) and Paula et al., (1996).

$$P_{solute} = kr/3 \quad (2)$$

Table 1. *Arabidopsis thaliana* mutants that were used in this study

Mutant	Lipid composition	Reference
fad 3-2	Reduction in desaturation of storage and membrane lipids; deficient in lineolate desaturase	Lemieux et al., 1990. Kunst et al., 1988
fad 7-2	Reduced glycerolipid n-3 desaturase, decrease in 16:3 and 18:3 fatty acids, increase in 16:2 and 18:2 fatty acids.	McConn et al., 1994
fad 7-1/fad 8-1	Reduction in desaturation of storage and membrane lipids, linoleic acid overproducer; linoleic acid underproducer.	Gibson et al., 1994 McConn et al., 1994
chs 1-1	Altered steryl-ester metabolism	Hugly et al., 1990
act 1-1	Chloroplast glycerol-3-phosphate acyltransferase	Kunst et al., 1989 Hugly et al., 1991

Time course changes in light scattering give two single exponential curves with opposite sign, the first one was very fast and corresponded to the exit of water from the vesicles, the second which has an opposite sign corresponds to the movement of the solute into the vesicles.

Influence of Lipid Composition and pH on Transmembrane Boric Acid Transport

Effect of Cholesterol on Permeability Coefficient

Liposomes were prepared as described above and cholesterol was added to result in cholesterol:phosphatidylcholine ratios of 0, 1:10, 2:10, 4:10, 6:10.

Effect of pH on Permeability Coefficient

Liposomes were prepared as described above with different buffers so that the final pHs of the buffer solution were 6.0, 7.0, 8.0, 9.0 and 10.0. The solutions contained 50 mM K_2SO_4 and 10 mM of the following buffers MES (pH = 6.0), PIPES (pH = 7.0), Tris-base (pH = 8.0), AMPSO (pH = 9.0), and AMP (pH = 10.0).

The fraction of undissociated boric acid was calculated according to the equation:

$$f_{H3BO3} = 1/(1 + (K_a/[H^+])) \qquad (3)$$

Where K_a is the dissociation constant of H_3BO_3, which H^+ is the proton concentration calculated from the pH of the medium. The fraction of $B(OH)_4^-$ can be calculated from: $f_{B(OH)4-} = 1 - f_{H3BO3}$.

Effect of Head Group

Phospholipids with different head groups including phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidic acid (PA) were purchased from Avanti Polar Lipids.

Effect of Acyl Chain Length

Different phosphatidylcholines with varying acyl chain lengths ranging from C16 to C24, as palmitoleyol, oleyol, eicosenoyl, erucol, nevronoyl were purchased from Avanti Polar Lipids (Paula et al., 1996).

Arabidopsis thaliana *Mutants Differing in Lipid Composition*

Five mutants and the wild type of *Arabidopsis thaliana* (ecotype Columbia) were obtained from the AIMS (Arabidopsis Information Management System, Colombus, OH) and were tested for differences in B uptake. The mutants were the following: *fad 3-2, fad 7-2, fad 7-1/fad 8-1, chs 1-1, act 1* and the wild type. The characteristics of the mutants used in this study are given in Table 1.

Germination

Seeds were surface sterilized with 70% ethanol and 10% bleach and placed at 4°C for two days to complete the dormancy period. Afterwards the seeds were germinated in petri dishes in MS medium with no hormones in the dark at 25°C then they were placed under continuous light and left to grow for 5 days. Then they were transferred onto a polyethylene net which was floated on nutrient solution according to Noguchi et al. (1997) with 50 μM of B as ^{11}B (99.51% Eagle Picher, Quapaw, OK, USA). The plants were grown for two weeks, then were harvested, dried, ashed and analyzed for B with the ICP-MS (Nyomora et al., 1997). Four replicates were used in this experiment.

Results

Figure 1 shows the light-scattering data obtained after mixing the liposomes made with egg phosphatidylcholine with 400 mM boric acid. The initial decrease in light scattering is due to the efflux of water when the vesicles were exposed to the higher external osmolarity. An increase in light scattering then occurred as a result of the movement of water and H_3BO_3 back into the liposomes. Both curves can be described by single exponential equations with opposite signs. The first change in light scattering was much faster and suggests a permeability to water of 3×10^{-3} cm sec^{-1}, the second portion of the curve corresponds to a permeability of boric acid of 4.9×10^{-6} cm sec^{-1}. Using this approach, the permeability of urea, glycerol, boric acid and water across phosphatidylcholine vesicles was determined. Each value is the average of 8–10 replications (Table 2).

Figure 2*a* shows the effect of changing cholesterol ratios on permeability of boric acid in liposomes. There

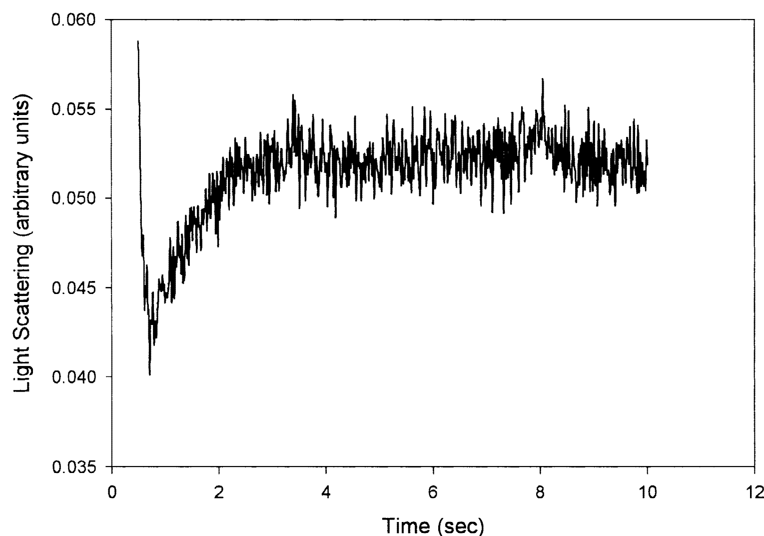


Fig. 1. Change in light-scattering intensity as a result of the exposure of liposomes to a transmembrane osmotic gradient following the addition of 400 mM of boric acid to the external solution.

Table 2. Permeability coefficient of boric acid, urea, glycerol and water across phosphatidylcholine liposome membranes at room temperature in solution with pH = 7

Compound	Permeability coefficient (cm sec^{-1})
H_3BO_3	$4.9 \pm 0.3 \times 10^{-6}$
Urea	$3.1 \pm 0.3 \times 10^{-6}$
Water	$3.5 \pm 0.5 \times 10^{-3}$
Glycerol	$4.3 \pm 0.1 \times 10^{-6}$

The values are mean of 8–10 replications with the standard error.

was a significant decrease in the permeability of liposomes to boric acid as the concentration of cholesterol increased to greater than 10%. Urea permeability was also affected by the presence of cholesterol in a manner similar to boric acid with a 95% decrease in permeability as the cholesterol fraction increased to 60% (Fig. 2*b*). Whereas 10% cholesterol reduced urea uptake by 40%, it had no effect on B uptake.

Changes in lipid head group had only a small effect on the permeability of boric acid (Fig. 3) with a small, but significant, decrease in permeability in the order, phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylinositol (PI), and phosphatidic acid (PA). The permeability observed with phosphatidic acid was about 30% lower than with phosphatidylcholine.

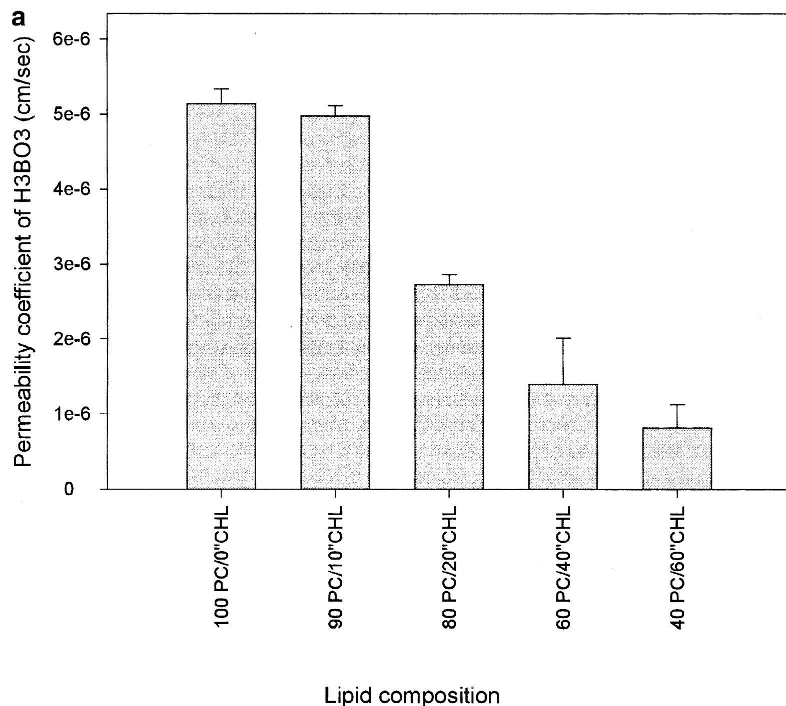
Figure 4*a* displays the effect of thickness of lipid bilayer (chain length) on the permeability of boric acid. There was a decrease in permeability with increasing bilayer thickness. The permeability coefficient for the shortest lipid (C16) was about 8-fold faster than for the longest lipid (C24). Similar results were observed for glycerol in which the permeability differed about 7-fold between longer and shorter lipid composition (Fig. 4*b*). Figure 5 shows the effect of lipid bilayer thickness on the permeation of boric acid, glycerol and H^+ (adapted from Paula et al., 1996). There was a decrease in permeability with increasing lipid bilayer thickness of about 80% for nonelectrolytes while for protons the change in permeability was much more substantial, decreasing by up to two orders of magnitude.

The effect of pH on the permeability of boric acid is displayed on Fig. 6. There was a significant reduction in permeability with increasing pH. At pH = 6 and 7, 99.9% of B is present as undissociated boric acid and the permeability of boric acid was 5×10^{-6} cm sec^{-1}. As pH increased there was a decrease in permeability and at pH = 9, P_{H3BO3} was 1.6×10^{-8} cm sec^{-1}. At this pH, 65% of the H_3BO_3 was undissociated while at pH = 10, 15% was present as undissociated boric acid and P_{H3BO3} was 9.5×10^{-9} cm sec^{-1}. This permeability decline of greater than 3 orders of magnitude indicates not only that borate ($B(OH)_4^-$) is not very permeable through the lipid bilayer but also suggests that pH directly affects permeability of H_3BO_3.

Significant difference in B uptake between the mutants of *Arabidopsis* compared with the wild type was observed. *Chs1-1* had the highest B uptake which was 30% higher compared with the wild type and 50% higher compared with the *act1-1* mutant. The *act1-1* mutant had the lowest total B uptake of all mutants tested and was 35% lower compared with the wild type. *Fad* mutants had generally lower uptake as *fad3-2* had 10%, *fad7-2* had 23% and *fad7-1/fad8-1* had 20% lower uptake (Fig. 7).

Discussion

One of the major constraints in determining the permeability coefficient of B is that it has no radioisotope, does



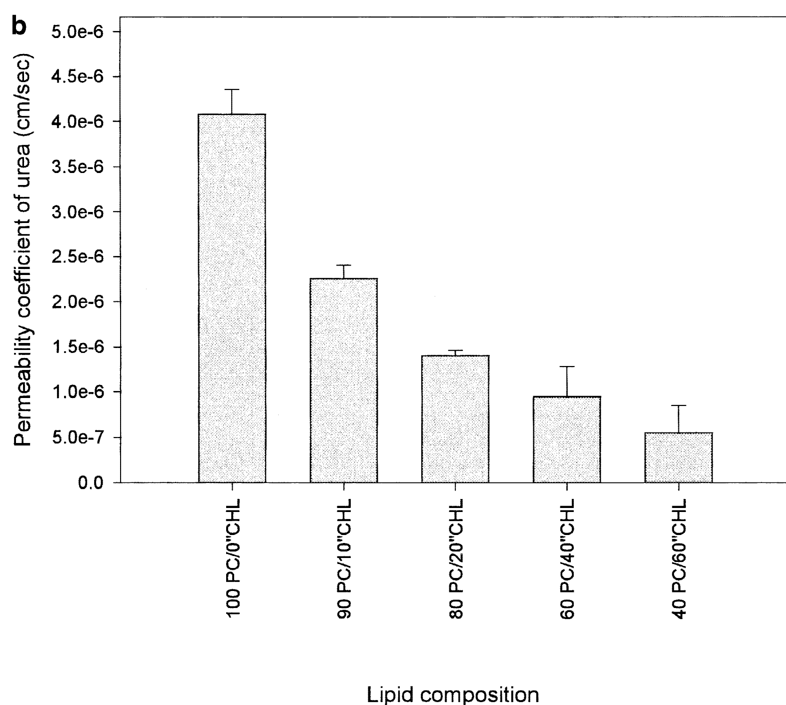


Fig. 2*a*. Permeability of H_3BO_3 as affected by changes in proportion of cholesterol in artificial liposomes. PC represents phosphatidylcholine and CHL represents cholesterol. Composition is expressed as mole % of total lipids.
b. Permeability of urea as affected by changes in proportion of cholesterol in artificial liposomes. PC represents phosphatidylcholine and CHL represents cholesterol. Composition is expressed as mole % of total lipids.

not complex with any described fluorescent probe and cannot be detected using electrical or pH shifts. To address this issue we have applied methodology that was developed for the determination of the permeabilities of urea and other nonelectrolytes.

The permeability coefficient of boric acid in PC liposomes was found to be 4.9×10^{-6} cm sec^{-1}, which is in good agreement with the predicted permeability coefficient calculated by Raven (1980) based on the ether/water partition coefficient. This permeability is close to the permeability measured for glycerol (4.2×10^{-6} cm sec^{-1}) and urea (3.1×10^{-6} cm sec^{-1}) and much slower than that of water (3×10^{-3} cm sec^{-1}). The permeability for water determined here agrees well with the P_f of planar lipid bilayers made from egg PC (the same lipid used here) which was determined to be 3×10^{-3} cm sec^{-1}

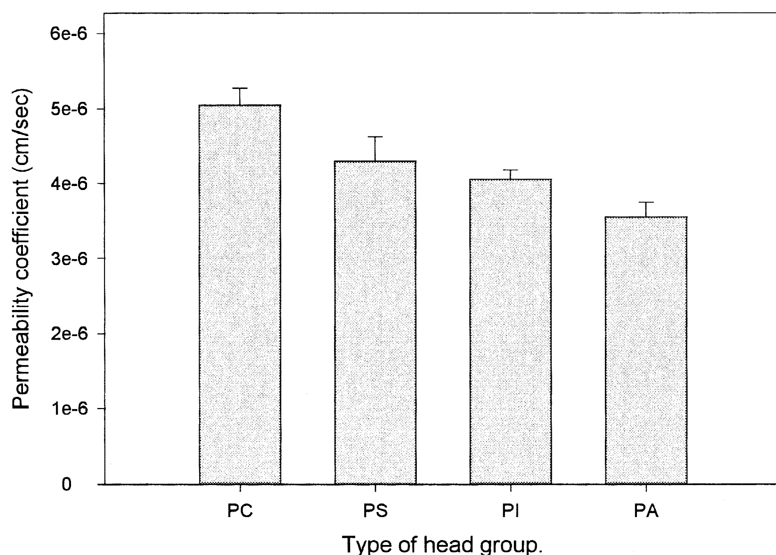


Fig. 3. Permeability of H_3BO_3 using different phospholipids with different head groups. PC stands for phosphatidylcholine, PS for phosphatidyl serine, PI for phosphatidylinositol and PA for phosphatidic acid.

at 25°C (Finkelstein, 1987). P_{urea} and $P_{glycerol}$ determined here agree well with those reported elsewhere (Verkman et al., 1985; Finkelstein, 1987). The agreement between our results and those reported elsewhere suggests that our method of measuring the permeability coefficient of nonelectrolytes and especially of boric acid is reliable.

Raven (1980) suggested that the theoretical permeability of plant membranes to H_3BO_3 would be the range of 10^{-5} to 10^{-6} cm sec^{-1}. Until now, however, the permeability of H_3BO_3 had not been determined in artificial lipid bilayers nor in plant membranes or plant cells. Though calculated partition coefficients in ether-water can correlate with measured permeability coefficients, examples of poor correlation between partition coefficient in ether-water and the measured permeability coefficient have been observed (Walter & Gutknecht, 1986). Small molecules with MW <50 show a 2–15-fold higher measured permeability than predicted (Walter & Gutknecht, 1986). The permeability of the small molecules was not solely correlated with the partition coefficient but it was more directly correlated with the molecular volume.

Boric acid has 3 hydroxyl groups and can form 6 hydrogen bonds with water. The activation energy of transport across a lipid bilayer can be calculated as 1.8 kcal $mole^{-1}$ per hydrogen bond, which suggests an energy of activity for H_3BO_3 of 10.8 kcal $mole^{-1}$ (Poznasky et al., 1976). Raven (1980) in another calculation, using the molecular weight of H_3BO_3 and the number of potential H-bonds that can form between H_3BO_3 and water molecules, and using a linear relationship between logPM1/2 and N (where P is the permeability coefficient, M the molecular weight and N the number of potential H-bonds), calculated a $P_{B(OH)3}$ of 4×10^{-6} cm sec^{-1}. Based on this result, Raven (1980) suggested that the passive plasma membrane P_{H3BO3} of a plant cell should be in the range of 10^{-6} to 10^{-5} cm sec^{-1}. This calculation did not take into account the possibility that lipid composition can significantly affect permeabilities, as has been observed for urea, glycerol, H^+, H_2O and other nonelectrolytes (Lande et al., 1994; Lande et al., 1995; Paula et al., 1996), and it can be expected to influence P_{H3BO3}.

Our data demonstrate that boric acid permeability follows the same pattern as other nonelectrolytes of its size such as urea and glycerol. The permeability coefficient calculated from the partition coefficient into water-ether (Raven, 1980) and the calculated permeability (based upon the size of boric acid and the number of H-bonds) are also in good agreement with the measured permeability from this study.

Mechanisms of Movement Through Lipid Bilayers

The mechanism of permeation of molecules across lipid bilayers is not clear. Two main mechanisms of permeation have been proposed: one is the solubility-diffusion mechanism where the permeating molecule is dissolved in the hydrophobic region, diffuses across it and then redissolves into the aqueous medium. The second mechanism is through hydrated transient defects which are caused by thermal fluctuations (Nichols & Deamer, 1980; Jansen & Blume, 1995; Volkov et al., 1997).

We used phosphatidylcholines with the fatty acid chain length ranging from 16 to 24 carbon atoms containing a variety of double bonds to assess the effect of chain length on H_3BO_3 permeability (Paula et al., 1996). We observed that there was a relatively small effect of thickness of the lipid bilayer on P_{H3BO3}, which agrees with the findings of Paula et al., (1996) for urea, glycerol

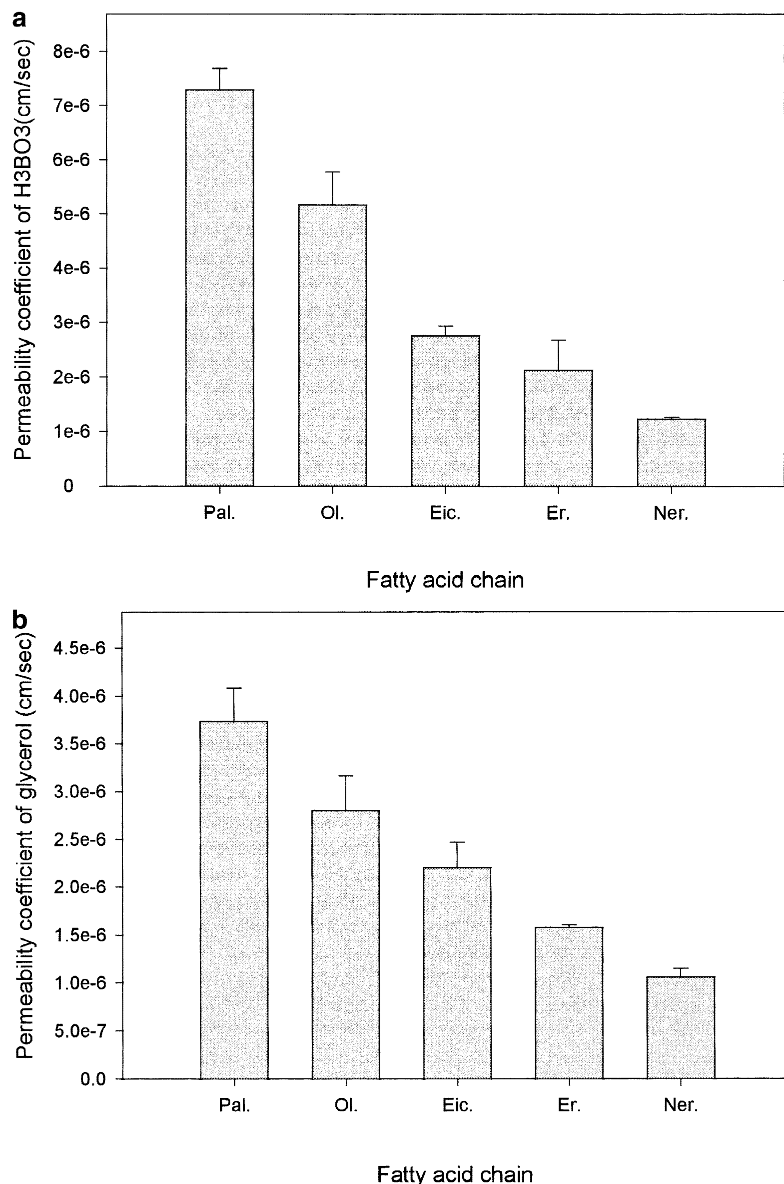


Fig. 4*a.* Permeability of H_3BO_3 as affected by chain length of phosphatidylcholines. In this experiment artificial phosphatidylcholines having different fatty acids were used. Where Pal. stands for palmitoleoyl, Ol. for oleoyl, Eic. for eicosenoyl, Er. for Erucoyl and Ner. for Nervonoyl. *b.* Permeability of glycerol as affected by chain length of phosphatidylcholines. In this experiment artificial phosphatidylcholines were used having different fatty acids. Where Pal. stands for palmitoleoyl, Ol. for oleoyl, Eic. for eicosenoyl, Er. for Erucoyl and Ner. for Nervonoyl.

and water. The results of Paula et al. (1996) suggest that the permeabilities of neutral solutes such as urea and glycerol by the pore mechanism would be very low. The solubility diffusion mechanism is therefore more favorable for the movement of the nonelectrolytes (such as undissociated H_3BO_3) than the pore mechanism. In contrast, it is likely that H^+ and cations are transported to a significant extent through transient defects in the membrane, since decreasing the thickness of lipid bilayers increases the number of pores and greatly increases permeability of these molecules (Paula et al., 1996).

Effect of Cholesterol and Head Group

Cholesterol has been shown to reduce the permeability of a wide range of nonelectrolytes, such as urea, water, glycerol and acetamide, and also of ions (H^+) (Lande et al., 1995). The reduction can be up to 9-fold, as was observed in our experiment (Lande et al., 1995). The effect of cholesterol on P_{H3BO3} and the permeabilities of other molecules likely occurs as a result of restraining the motion of lipid chains and the increase in head group mobility (Saito et al., 1991; Hanes & Liebovitch, 1995). Cholesterol also reduces the amount of water in the hydrocarbon domain (Haines & Liebovitch, 1995), reducing the membrane fluidity which causes a reduction in the rate of solute movement across the bilayer (Lande et al., 1995).

It has been speculated that cholesterol enhances membrane mechanical coherence and suppresses the passive transmembrane permeability in eukaryotic plasma cell membranes (Mouritsen et al., 1995). Our data agree

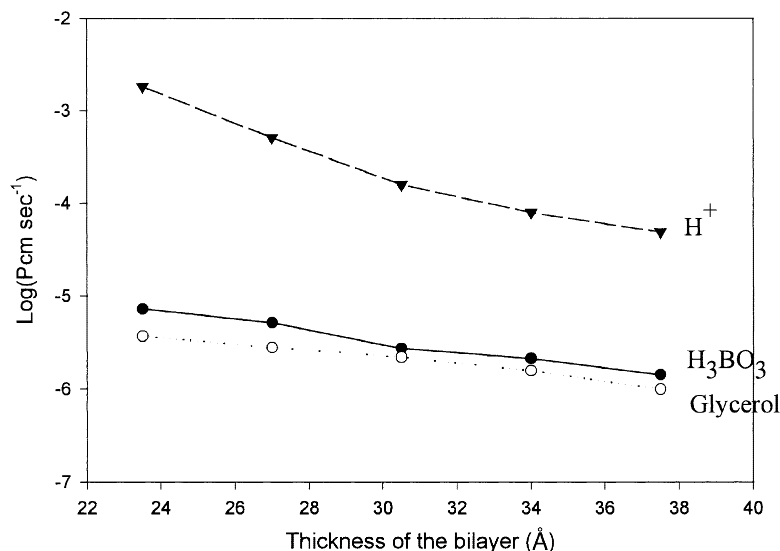


Fig. 5. Effect of the thickness of the bilayer on H_3BO_3, glycerol and H^+ permeability on a semilogarithmic scale. Permeability coefficients of H_3BO_3 and glycerol were measured from this experiment, and H^+ permeability data were obtained from Paula et al. (1996).

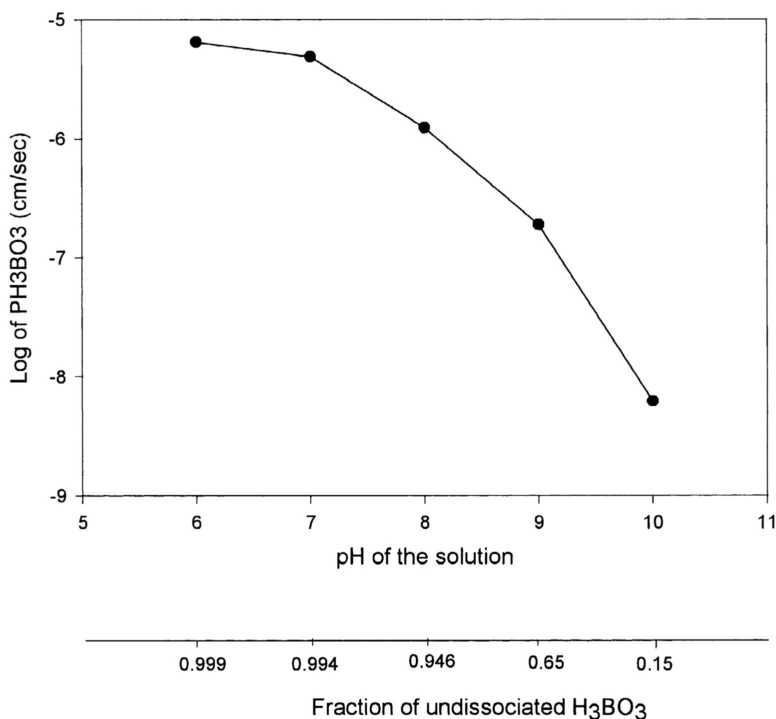


Fig. 6. Effect of pH on the permeability of H_3BO_3 across phosphatidylcholine liposomes.

with this report and others that found that bilayers having a cholesterol/phospholipid ratio of 1:4 can cause an 80% reduction in permeability of water and nonelectrolytes relative to membranes made purely of phosphatidylcholine (PC) (Lande et al., 1995).

In higher plants, several sterols, including sitosterol, stigmasterol, and 24 g-methylcholesterol (which is a mixture of campesterol and dihydrobrassicatesterol), are found in the plasma membrane (Hartman & Benveniste, 1987). The Δ5 double bond of cholesterol is the most effective conformation for optimal sterol-phospholipid interactions and regulation of membrane permeability (Ranadive & Lola, 1987). Schuler et al., (1991) found that sitosterol and 24γ-methylcholesterol reduced significantly water permeability, which could be because these sterols stabilize the lipid bilayer. In contrast, stigmasterol had no significant effect on the swelling rate of soybean phosphatidylcholine vesicles, while two unusual sterols, 24γ-methylpollinosterol and 14α, 24γ-dimethylcholesterol-8-en-3b-ol cycloartenol (another cyclopropylsterol), had the strongest effect in reducing the permeability of water among all the other sterols (including

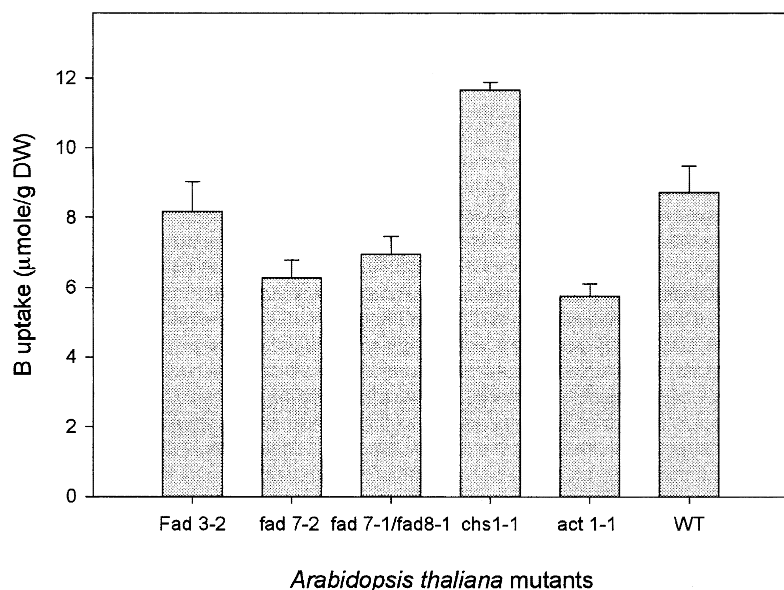


Fig. 7. Boron uptake of six *Arabidopsis thaliana* mutants and the wild type having different lipid composition in the plasma membrane and grown in hydroponic solution.

cholesterol). This indicates that some of the sterols found in plants can have the same or even stronger effect in reducing membrane permeability for boric acid or other nonelectrolytes. It has been speculated that differences in B uptake between cultivars or species can be due to differences in membrane permeability and that this could be due to differences in sterol content or distribution.

Effect of pH

The data reported in this paper show that boric acid permeability across lipid bilayers is dependent on the pH. Chakrabarti et al. (1992) found that the permeability of lysine methyl ester was pH dependent. In contrast Chakrabarti and Deamer (1992) found that pH had a little effect on permeability of amino acids (glycine, lysine, serin tryptophan and phenylalanine).

Chakrabarti et al. (1992), Gutkneckt and Walter (1981) showed that the permeability of charged molecules is much less compared to that of neutral forms, as the charged group prevents the molecule from diffusing across the hydrophobic bilayer. The fact that pH has little effect on permeability of amino acids in the study reported by Chakrabarti and Deamer (1992) indicates that these amino acids probably cross the lipid bilayer through pores (transient defects) in the membrane or that amino acids are charged at all pHs.

There was a sharp decline in the apparent permeability measured for both species present (H_3BO_3, $B(OH)_4^-$) as the pH increased, which indicates that H_3BO_3 is much more permeable than $B(OH)_4^-$. The much greater decrease in the permeability (>99%) at pH = 9.25 compared with the decrease in the fraction of undissociated boric acid (which was 50%) indicates that the relationship between permeability and pH is not linear and that the 50% change in the fraction of undissociated boric acid does adequately explain the change in permeability. Similar results were observed by others (Gutknecht & Walter, 1981; Chakrabarti et al., 1992; Xiang & Anderson, 1994), who explained this difference between the decrease in the percentage of undissociated form of the molecules and the much higher decrease in permeability as indicating that the charged form does not cross the membrane or it crosses in a very low rate.

Arabidopsis Mutants

Chs1-1 mutant is a chilling sensitive mutant and has 10-fold increase in the amount of steryl esters while the concentration of the polar and neutral lipids is not different from the wild type (Hugly et al., 1990). Under control conditions, the *chs1-1* mutant had 20% less sterols compared with the control, the wild type (Hugly et al., 1990). The decrease in sterols in chl1-1 increases the fluidity of the membrane, which can affect the diffusion of molecules through the lipid bilayer. Increasing the sterol content is known to decrease the permeability of water, and nonelectrolytes such as urea, acetamide and glycerol and was observed here (Schuler et al., 1991; Lande et al., 1995).

Fad3-2 has increased levels of 18:3 in roots resulting in a ratio of 18:3/18:2 that is 3 times higher compared with the wild type plants, while the proportion of the other fatty acids were unchanged (Shah et al., 1997). The increased amount of unsaturated fatty acids could explain the 10% increase in B uptake observed here since unsaturation can increase membrane fluidity and permeability of uncharged molecules (Lynch & Steponkus, 1987).

Fad7-1/fad8-1 is also a desaturation defective mutant and has a much lower concentration of unsaturated fatty acids than the wild type (McConn et al., 1994). The changes in lipid composition resulted in decreased B uptake possibly as a result of the increased length of the fatty acids in the mutants.

Actl-1 is a mutant which is defective in the enzyme glycerol-3-phosphate acyltransferase which converts a lipid 16:3 into 18:3 lipid (Kunst et al., 1989). Both of these changes in the lipid composition are found in the general fraction of lipids and are likely present in the plasma membrane. The decrease in permeability observed with increasing fatty chain length was observed also in artificial liposomes (Paula et al., 1996).

In nutrient uptake studies it is possible that differences in total nutrient uptake can occur due to differences in plant growth and overall nutrient accumulation. In addition to the experiments conducted here we also conducted several preliminary short-term uptake experiments (1, 2, 4 hours) were conducted and indicated that lipid composition significantly influenced B uptake in a manner consistent with results from artificial vesicles reported above. Subsequent experimentation in which uptake was determined over a 1, 2 and 4 days uptake period further verified this observation (*data not shown*). In these experiments no difference in plant growth was observed between the *Arabidopsis* mutants suggesting that differences in the total B uptake that was reported in this study could be attributed to the effect of lipid composition on the permeability of boric. The results of all uptake experiments were consistent irrespective of uptake period.

In this study we reported that lipid composition can significantly affect B uptake. Preliminary data from our group also suggest that aquaporins may also contribute to B uptake in plants which is consistent with observations in animal systems in which aquaporins are known to be involved in the transport of non electrolytes such as urea and glycerol which share several physical and chemical similarities to boric acid (Agre et al., 1998).

In conclusion the permeability coefficient of boric acid for artificial membranes is very close to the predicted permeability coefficient based upon ether/water partition coefficient. Permeability is affected by lipid composition such as head group, cholesterol and fatty acid chain length. pH had the strongest effect on permeability, reducing it by 3 orders of magnitude as pH increased from 6 to 10. Boric acid seems to behave like urea and is affected by similar factors, suggesting it permeates through the lipid bilayer through a solubility diffusion mechanism. Since the permeability of boric acid is quite high (4.9×10^{-6} cm sec^{-1} in phosphatidylcholine vesicles), passive transport across plant membranes could be an important mechanism of B uptake, particularly when B is present at an adequate level in the external medium. Also we found that changes in lipid composition can affect the permeability and subsequent uptake of B in plants. It is possible that differences in B uptake are due to the differences in lipid composition of the plasma membrane of plant cells. It still remains unclear if differences in B uptake between cultivars can be wholly ascribed to differences in lipid composition.

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